MISSOURI

BOTANICAL GARDEN

TWENTY-FIRST ANNUAL REPORT

ST. LOUIS, MO.
PUBLISHED BY THE BOARD OF TRUSTEES
1910

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A. D. CUNNINGHAM, Secretary.

******Ex-Officio.*

[1] Elected President of the Board in November, 1910, to succeed David F. Kaime, who had succeeded Rufus J. Lackland in that office early in the year, and occupied the office at the time of his death.

[2] Elected Vice-President of the Board in November, 1910, to fill a vacancy caused by the election of Mr. Kaime to the presidency on the resignation of this office by Mr. Lackland very shortly before his own death.

[3] Elected to membership in November, 1910, to fill a vacancy caused by the death of Rufus J. Lackland, one of the Trustees designated by Mr. Shaw.

[4] Elected President of the School Board in October, 1910, to succeed Robert Moore, who had held that office for one year.

STAFF
OF THE MISSOURI BOTANICAL GARDEN.

Director,
WILLIAM TRELEASE.

GEORGE T. MOORE,
Plant Physiologist.

HERMANN VON SCHRENK,
Plant Pathologist. *

REGINALD R. GATES,
Research Assistant.

CORA J. HOGAN,
Curator of Library.

MOSES CRAIG,
Curator of Herbarium.

HENRY C. IRISH,
Superintendent.

JAMES GURNEY,
Head Gardener. †

JOHN BANNES,
Foreman. †

OTTO BOGULA,
Foreman.

HARRY W. ANDERSON,
CHARLES O. CHAMBERS,
STOCKTON M. MCMURRAN,
CAROLINE RUMBOLD,
JACOB SCHRAMM,
Rufus J. Lackland Research Fellows.

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* *Honorary.* † *Emeritus.*

PREFACE.

Under direction of the Board of Trustees, the twenty-first annual report of the Missouri Botanical Garden is presented to the public.

The twentieth report, of 1909, was not issued until January 14, 1910, because of unavoidable delays in press and bindery; but separates of Dr. Widmann's paper had been issued early in the season, those of Dr. Griffiths' paper on March 22d, and those of the other scientific papers on December 31st.

These reports are sent to scientific institutions and journals in exchange for publications and specimens desirable for the library, herbarium, laboratories or living collections of the Garden. So far as is possible, reprints of the botanical articles they contain are sent to botanists occupied with a study of the subjects to which they pertain.

Any of the Garden publications not out of print may be purchased, at approximately the cost of publication, from Messrs. R. Friedländer & Sohn, Berlin, Germany; W. Wesley & Son, London, England; or the undersigned.

WILLIAM TRELEASE.

ST. LOUIS, MO., November 15, 1910.

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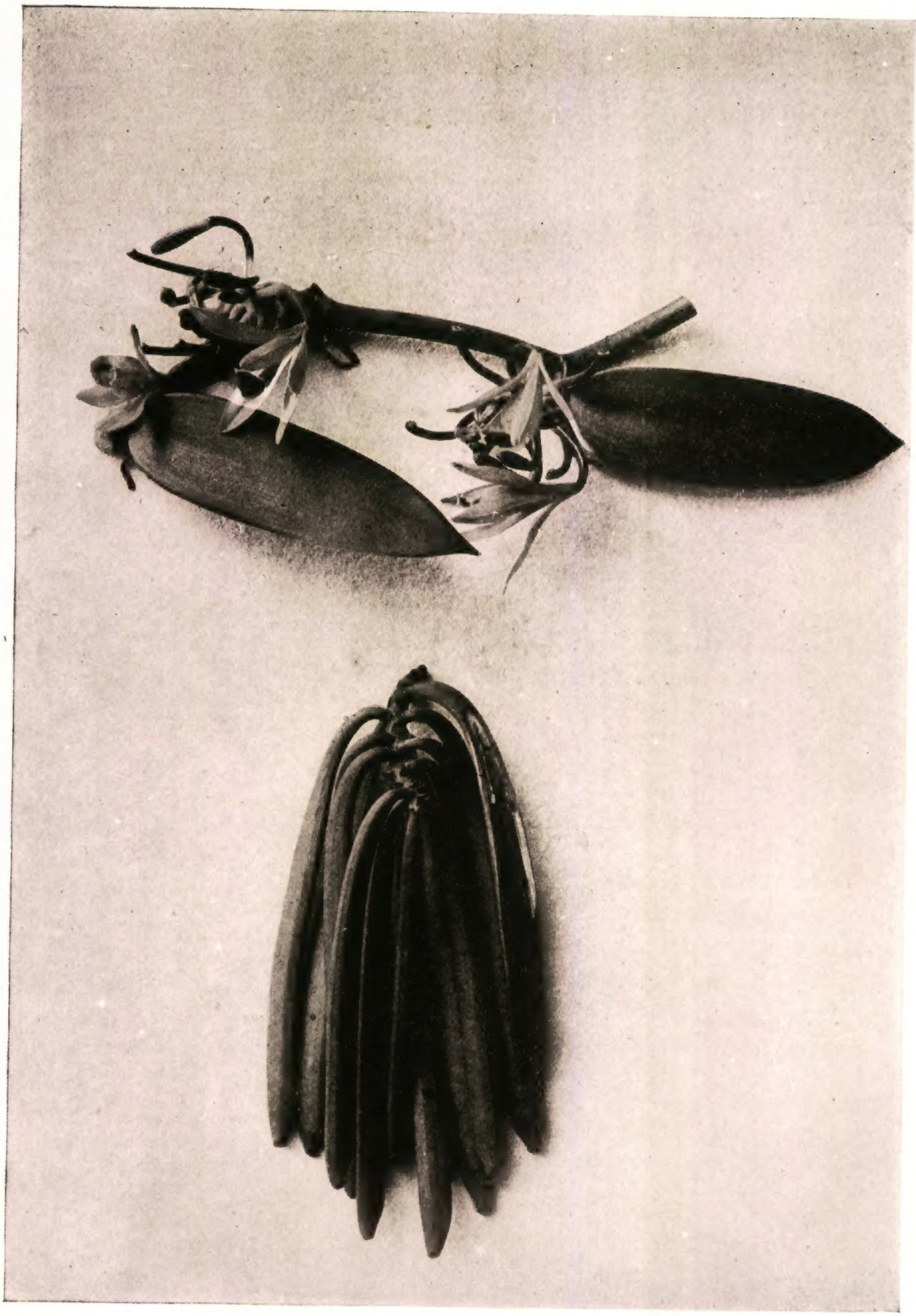


VANILLA FLOWERS AND FRUIT.

THE LIBRARY AND LABORATORIES.

REPORTS FOR THE YEAR 1909.

REPORT OF THE OFFICERS OF THE BOARD.

SUBMITTED TO THE TRUSTEES JANUARY 12, 1910.

To the Board of Trustees of the Missouri Botanical Garden:

We submit for your consideration the financial results for the year ending December 31, 1909.

Our receipts from rentals were $2,322.32 in excess of those for the year 1908, in addition to which our taxes were $3,534.02 less than the year previous, that amount having been assumed by the lessee of one piece of property as part of the rental, so that the actual increase was $5,866.34. Our loss through vacancies during the year amounted to $2,620.68, and $282.50 was charged off as uncollectable,—about one-fifth of 1% on the total earnings. We have several warehouses vacant at this time, but we do not anticipate any diminution in our income during the ensuing year.

No improvements to the income property have been made other than necessary repairs.

We have disposed of 178 feet on Flora boulevard, for the sum of $13,650.00; and 210 feet on McRee avenue between Grand and Spring avenues, for $7,316.87.

Demands for street improvements have not been so heavy as in recent years, but the following expenditures have been made and charged to real estate as a betterment:

Lawrence street	Roadway and sidewalks . .	$ 2,885 79
Thurman avenue	Sidewalks	382 64
Tower Grove avenue . . .	Sidewalks	957 76
Spring avenue	Sidewalks	1,033 96
Sewer between McRee and Lafayette avenues		7,440 27
		$12,700 42

Several streets just completed or under contract will call for additional expenditures during the year 1910, as follows:

some of which were anticipated in our last report, but completion of the improvements was delayed:

Shaw avenue	3,112 front feet	$18,672 00
Klemm street	2,000 front feet	10,000 00
Vandeventer avenue	2,000 front feet	8,500 00
Sidewalks	3,000 front feet	3,000 00
		$40,172 00

The library and laboratory building at the Garden, mentioned in our last report, has been completed at a total cost of $54,593.82 and the sum of $6,793.80 has been expended for metal book and herbarium cases, etc. No other expenditures have been made at the Garden other than for its maintenance, at a total cost of $54,145.28.

The following amounts have been credited to the Stock Account for the year:

Library	$ 5,468 73
Herbarium	5,543 85
Library building and furnishings	61,387 62
	$72,400 20

The annual bequests provided for in Mr. Shaw's will, with the exception of the Trustees' Banquet, have been met by the expenditure of $766.50, but the full amounts authorized, $2,100.00 have been charged against the year's income, to be used at some future time.

RECEIPTS.

Receipts on account of rentals	$139,591 36	
Interest and dividends	1,370 10	
Garden handbook sales	166 00	
Publication sales	4 50	
Garden tuition	19 20	
Total income receipts		$141,151 16
Sales of real estate under decree	20,966 87	
Shaw School of Botany, rent	3,900 00	
Bills receivable	4,360 00	
	29,226 87	
Total receipts		$170,378 03
Cash on hand December 31, 1908		4,070 34
		$174,448 37

DISBURSEMENTS.

Garden Account,		
Labor pay-roll	$22,966 80	
Students' pay-roll	2,152 32	
Office assistance	1,256 25	$26,375 37
Fuel		1,904 80
Water		557 00
Repairs and supplies	2,693 56	
Stable and implements	364 54	
Plants and seeds	759 78	$32,655 05
Herbarium Account,		
Salaries	1,080 00	
Fuel	419 99	
Current expense	1,833 92	3,333 91
Library Account,		
Salaries	2,220 57	
Fuel	518 73	
Current expenditures	2,872 30	5,611 60
Office Account,		
Salaries	5,796 51	
Fuel	273 03	
Current expenditure	470 30	6,539 84
Research and instruction,		
Salaries	4,087 42	
Current expense	172 18	
Furnishing laboratories	1,745 28	6,004 88
Total maintenance		$54,145 28
Garden Improvements,		
Library building, balance for completion	14,253 62	
Renovating old library building	457 33	
Metal cases for library and herbarium	6,793 80	21,504 75
Total expended on the Garden		$75,650 03
Publication Account,		
Twentieth annual report	1,905 00	
Seventh report, reprint	380 00	2,285 00
Carried forward		$77,935 03

Brought forward		$77,935 03
Property Account,		
State, school, city and sprinkling taxes	$33,701 50	
Streets, sidewalks and sewers . . .	15,158 32	
Insurance	3,974 59	
Repairs	7,608 27	
Improvements	300 00	60,742 68
Bequests,		
Annual Flower Sermon	200 00	
Annual Flower Show	396 00	
Annual Gardeners' Banquet	170 50	766 50
Sundries,		
Office expenses	6,680 37	
Legal and professional expenses . .	268 70	
Commission	2,341 25	9,290 32
Bonds, stocks and certificates		14,747 11
Shaw School of Botany, rents		3,936 75
Total Disbursements		$167,418 39
Cash on hand December 31, 1909 .		7,029 98
		$174,448 37

Respectfully submitted,

R. J. LACKLAND, President.

Attest:

A. D. CUNNINGHAM, Secretary.

A SAUROMATUM.

TWENTY-FIRST ANNUAL REPORT OF THE DIRECTOR.

SUBMITTED TO THE TRUSTEES JANUARY 12, 1910.

To the Board of Trustees of the Missouri Botanical Garden:

The following report on the Missouri Botanical Garden and the School of Botany therewith connected is respectfully submitted, in compliance with your rules.

GARDENING.

Decorative gardening in 1909 followed essentially the same lines as for several years past, the most observable changes consisting in modification of the borders flanking the entrance, in the substitution of foliage for flowering plants in the sunken garden, and in the use of more than the usual number of succulents in attractive and skillful mosaic designs. In the early spring the sunken garden and its surroundings were devoted to tulips, as has been the case for several years past, and the display of these flowers was extended by planting additional beds with the brilliant, late-blooming "parrot" varieties. Aside from the tulips, of which 28,800 plants representing 222 varieties were used, the bedding plants placed during the season numbered 33,000.

Chrysanthemums were again grown in numbers, and 3,511 plants, of 520 varieties, were shown under canvas through the fortnight beginning with November 15th. Though the season had been less favorable than usual for the growth of these plants, and the specimen plants were not as large as in some earlier years, a very creditable display was made, and in variety of instructive forms this collection has not before been equaled.

About 2,500 Oenotheras, grown from pedigree-seed, added much to the attractiveness of the Garden in the early evening during the summer, because of their profuse production of large and fragrant flowers, though they were intended primarily for research use.

Plant and seed accessions, apart from those propagated or collected at the Garden, number 326, and comprise 16,433

plants or packets of seeds of which 9,960, corresponding to 31 accession entries, were purchased at a cost, including transportation charges, of $759.78; 6,473, representing 295 of the accessions, valued at $694.82, were presented; and 3,271, or 39 accessions, valued at $194.03, were collected by Garden employees—in addition to a large number of seeds for exchange purposes. Garden propagations amounted to 23,618 plants, valued at $1,653.26; and 13,250 seedlings, valued at $662.50, were also raised by the gardeners.

The exchange seed list issued by the Garden last winter included 2,369 species or named varieties; and 8,159 packets of seeds, valued at $407.95, selected from the list have been issued to correspondents. Living plants to the number of 169, valued at $16.30, have been similarly distributed. In addition to these exchanges 207 duplicate plants, valued at $26.20, have been presented to schools and colleges for educational use, and 1,133 surplus plants, removed from the grounds on the approach of winter or remaining after the spring planting, were given to schools and playgrounds. Chrysanthemum plants which were still usable, numbering 208, and many cut flowers from others, were distributed to hospitals and similar charities after the November exhibition.

PLANTS CULTIVATED.

During the year 822 species or varieties were added to the collection, and 520 lost or discarded, leaving a net recorded gain of 302, and bringing the total at the end of the year to 11,764, as compared with the 11,464 noted for 1908.[1] These forms represent 1,777 genera, belonging to the following 197 families of plants:

Families	Genera	Species	Families	Genera	Species
Acanthaceae	14	35	Anacardiaceae	8	32
Aceraceae	2	36	Anonaceae	3	6
Agaricaceae	1	5	Apocynaceae	17	34
Alismaceae	3	10	Aponogetonaceae	1	1
Aizoaceae	4	16	Aquifoliaceae	2	10
Amarantaceae	14	45	Araceae	32	254
Amaryllidaceae	25	366	Araliaceae	9	36

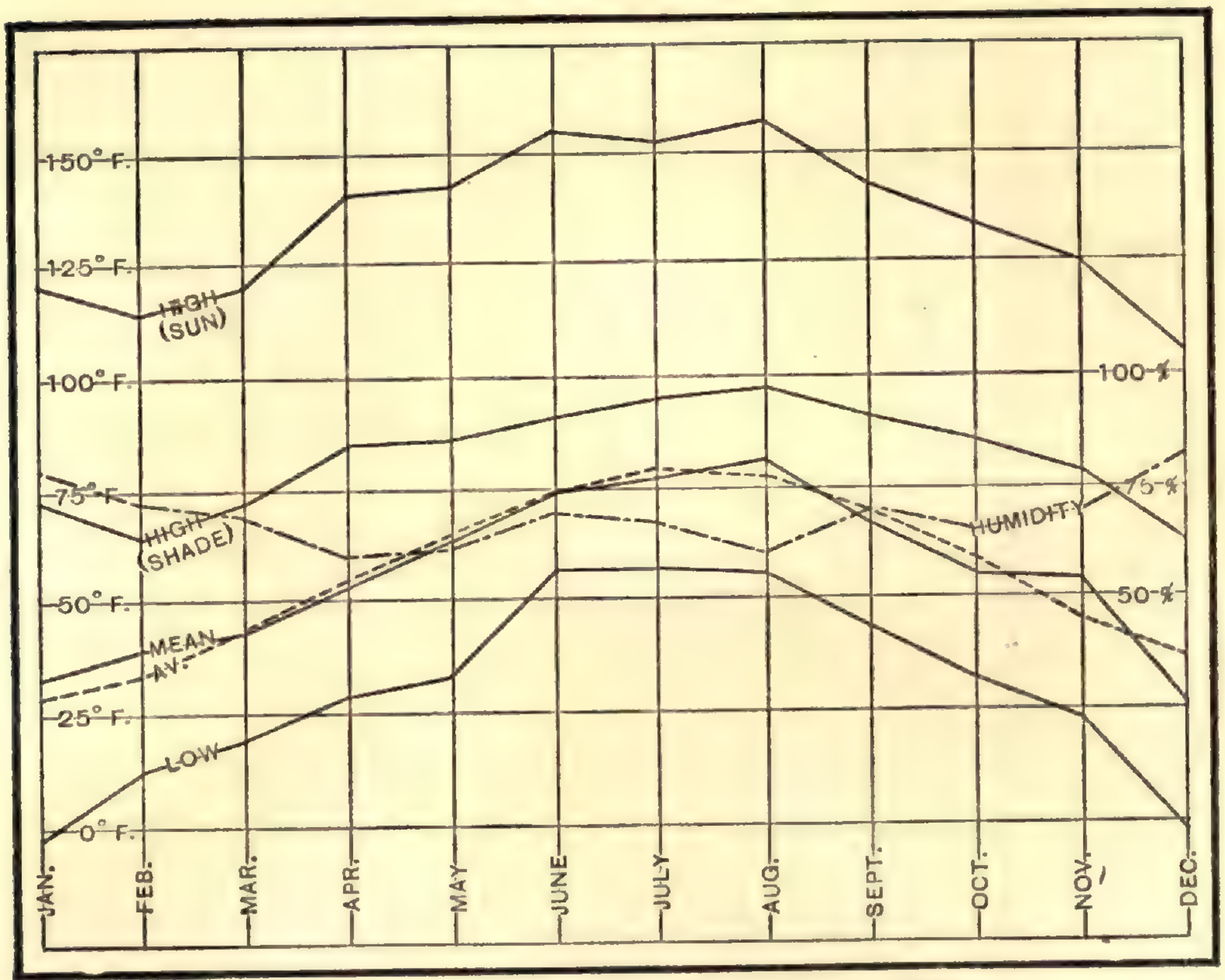
[1] Rept. Mo. Bot. Gard. 20: 15.

Families	Genera	Species	Families	Genera	Species
Aristolochiaceae	2	16	Dilleniaceae	4	4
Asclepiadaceae	20	114	Dioscoreaceae	1	8
Balsaminaceae	1	8	Dipsacaceae	4	29
Begoniaceae	1	51	Droseraceae	2	4
Berberidaceae	5	33	Ebenaceae	3	7
Betulaceae	5	41	Elaeagnaceae	3	12
Bignoniaceae	17	55	Empetraceae	1	1
Bixaceae	1	1	Equisetaceae	1	3
Bombaceae	6	7	Ericaceae	13	52
Borraginaceae	19	40	Eriocaulaceae	1	1
Bromeliaceae	15	122	Euphorbiaceae	23	158
Brunelliaceae	1	2	Fagaceae	3	50
Burseraceae	2	2	Filicineae	44	187
Butomaceae	2	2	Flacourtiaceae	4	5
Buxaceae	3	6	Fouquieriaceae	1	2
Cactaceae	21	603	Gentianaceae	3	5
Calycanthaceae	2	5	Geraniaceae	3	94
Campanulaceae	10	76	Gesneriaceae	15	108
Cannaceae	1	215	Ginkgoaceae	1	1
Capparidaceae	4	9	Gnetaceae	1	2
Caprifoliaceae	8	86	Gramineae	84	301
Caricaceae	1	3	Guttiferae	8	35
Caryophyllaceae	12	125	Haemodoraceae	2	2
Casuarinaceae	1	6	Halorrhagidaceae	2	2
Celastraceae	4	25	Hamamelidaceae	3	3
Chenopodiaceae	12	60	Hippocastanaceae	1	7
Chloranthaceae	1	1	Hydrocharitaceae	3	4
Cistaceae	3	24	Hydrophyllaceae	5	11
Clethraceae	1	1	Icacinaceae	1	1
Cneoraceae	1	1	Iridaceae	18	232
Cochlospermaceae	1	1	Juglandaceae	3	16
Combretaceae	3	8	Juncaceae	3	22
Commelinaceae	9	28	Juncaginaceae	1	2
Compositae	121	1260	Labiatae	43	173
Convolvulaceae	7	26	Lardizabalaceae	2	2
Coriariaceae	1	1	Lauraceae	10	12
Cornaceae	3	24	Lecythidaceae	3	3
Corynocarpaceae	1	1	Leguminosae	117	537
Crassulaceae	20	160	Leitneriaceae	1	1
Cruciferae	30	173	Lemnaceae	1	1
Cucurbitaceae	19	91	Lentibulariaceae	1	1
Cycadaceae	6	29	Liliaceae	77	792
Cyclanthaceae	1	1	Linaceae	2	18
Cyperaceae	10	56	Loasaceae	3	3
Cyrillaceae	1	1	Loganiaceae	5	13
Diapensiaceae	1	1	Loranthaceae	1	1

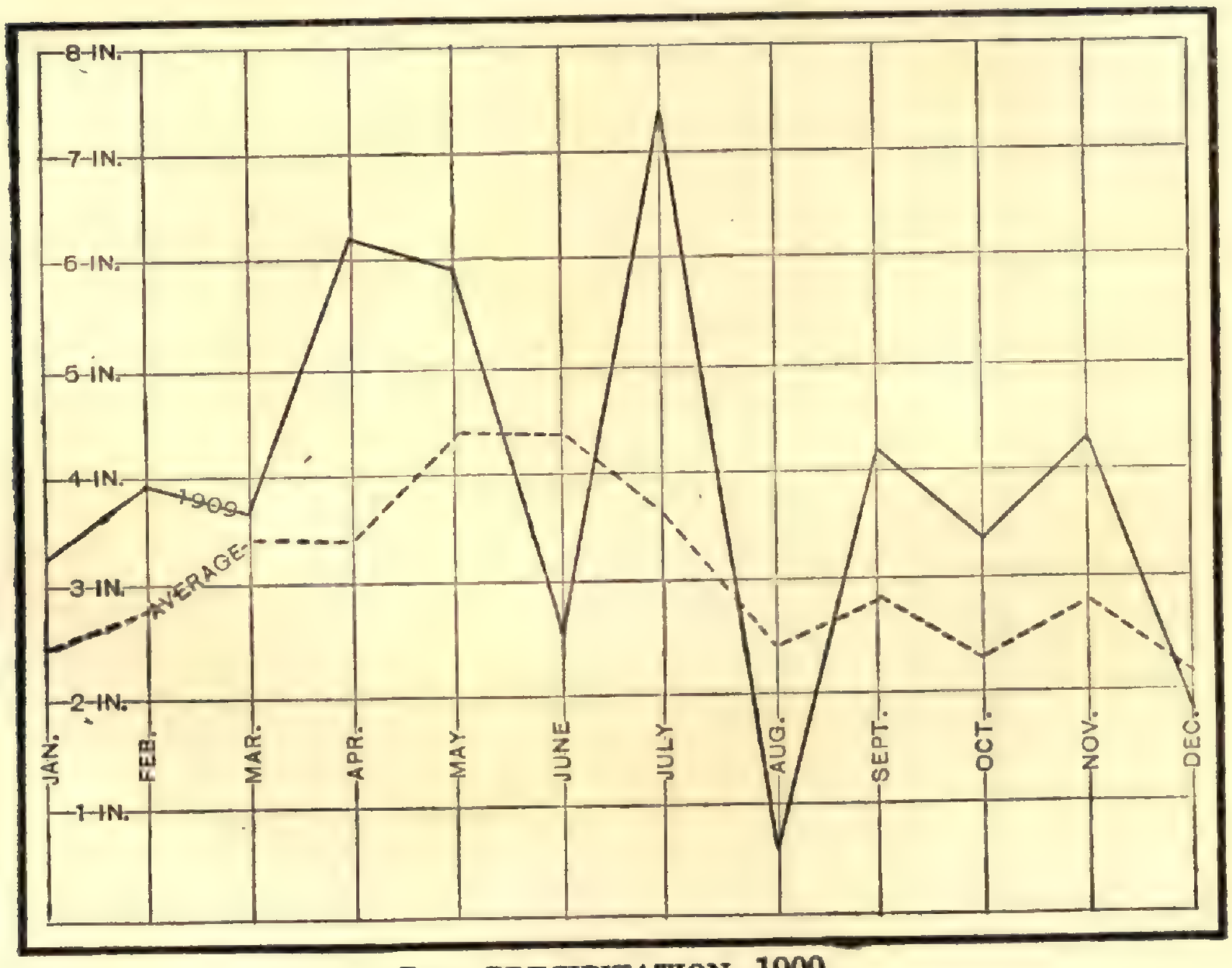
Families	Genera	Species	Families	Genera	Species
Lycopodiaceae	1	1	Proteaceae	3	6
Lythraceae	6	21	Punicaceae	1	2
Magnoliaceae	5	23	Ranunculaceae	20	285
Malpighiaceae	6	8	Resedaceae	3	13
Malvaceae	22	96	Rhamnaceae	11	37
Marantaceae	4	24	Rosaceae	34	876
Marchantiaceae	6	10	Rubiaceae	31	66
Marsileaceae	2	3	Rutaceae	16	32
Martyniaceae	2	8	Salicaceae	2	81
Melastomaceae	6	8	Salvadoraceae	1	1
Meliaceae	5	7	Salviniaceae	1	1
Melianthaceae	1	2	Santalaceae	2	2
Menispermaceae	6	9	Sapindaceae	14	20
Moraceae	16	64	Sapotaceae	7	12
Moringaceae	1	1	Sarraceniaceae	1	5
Musaceae	3	14	Saururaceae	2	2
Myricaceae	1	4	Saxifragaceae	16	96
Myrsinaceae	3	12	Scrophulariaceae	24	114
Myrtaceae	18	98	Selaginellaceae	1	7
Nepenthaceae	1	32	Simarubaceae	1	1
Nolanaceae	1	4	Solanaceae	22	262
Nyctaginaceae	3	10	Sparganiaceae	1	1
Nymphaeaceae	6	61	Staphyleaceae	1	4
Ochnaceae	1	1	Sterculiaceae	8	19
Oleaceae	10	135	Styracaceae	2	7
Onagraceae	9	61	Symplocaceae	1	1
Orchidaceae	99	640	Taccaceae	1	3
Oxalidaceae	3	45	Tamaricaceae	1	3
Palmae	39	86	Taxaceae	2	8
Pandanaceae	1	11	Theaceae	5	14
Papaveraceae	13	80	Theophrastaceae	1	1
Passifloraceae	1	7	Thymelaeaceae	3	3
Pedaliaceae	1	1	Tiliaceae	6	20
Phytolaccaceae	5	12	Tropaeolaceae	1	6
Pinaceae	16	101	Typhaceae	1	4
Piperaceae	2	12	Ulmaceae	4	12
Pittosporaceae	3	19	Umbelliferae	38	111
Plantaginaceae	1	23	Urticaceae	10	25
Platanaceae	1	4	Valerianaceae	3	5
Plumbaginaceae	4	29	Verbenaceae	12	80
Polemoniaceae	3	35	Violaceae	1	32
Polygalaceae	1	3	Vitaceae	3	66
Polygonaceae	12	63	Zingiberaceae	9	23
Pontederiaceae	3	4	Zygophyllaceae	2	2
Portulacaceae	5	25			
Potamogetonaceae	1	3	Totals....197	1,777	11,764
Primulaceae	7	57			

GARDEN-GROWN VANILLA.

DIAGRAMS A AND B.



A. — TEMPERATURE AND HUMIDITY, 1909.



B. — PRECIPITATION, 1909.

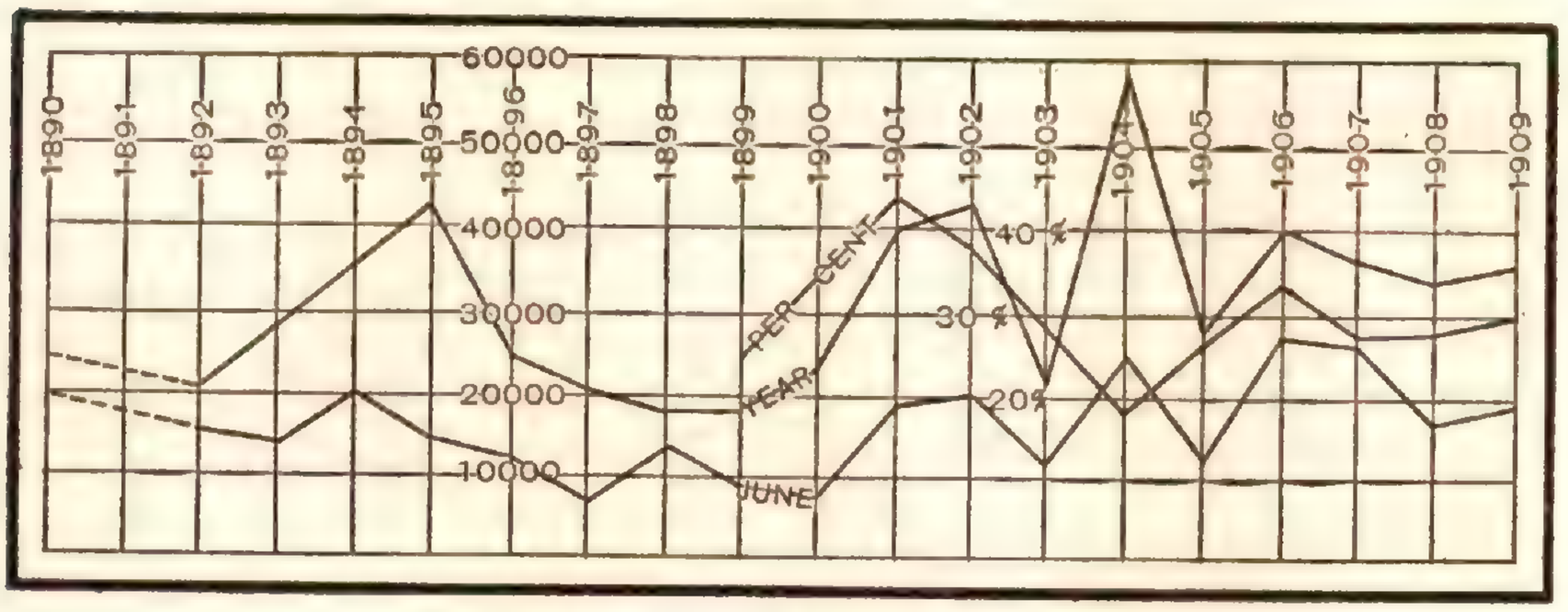
THE WEATHER.

Though January, February and November, and to a less extent August, were this year markedly warmer, and December decidedly colder, than usual, the mean temperature for the season (Diagram A) closely follows the average, except for the fall and winter months. The precipitation for the year (Diagram B) has been rather abundant (47.5 in.); but the rainfall in June was considerably below the average and less than an inch of rain fell in August, which, with the rather high temperature, increased the difficulties of gardening and especially affected the blue-grass lawns. The diagrams are derived from the local Weather Bureau records.

VISITORS.

A gratifying number of visitors is to be reported. As may be seen from the accompanying diagram, they closely agreed

DIAGRAM C.



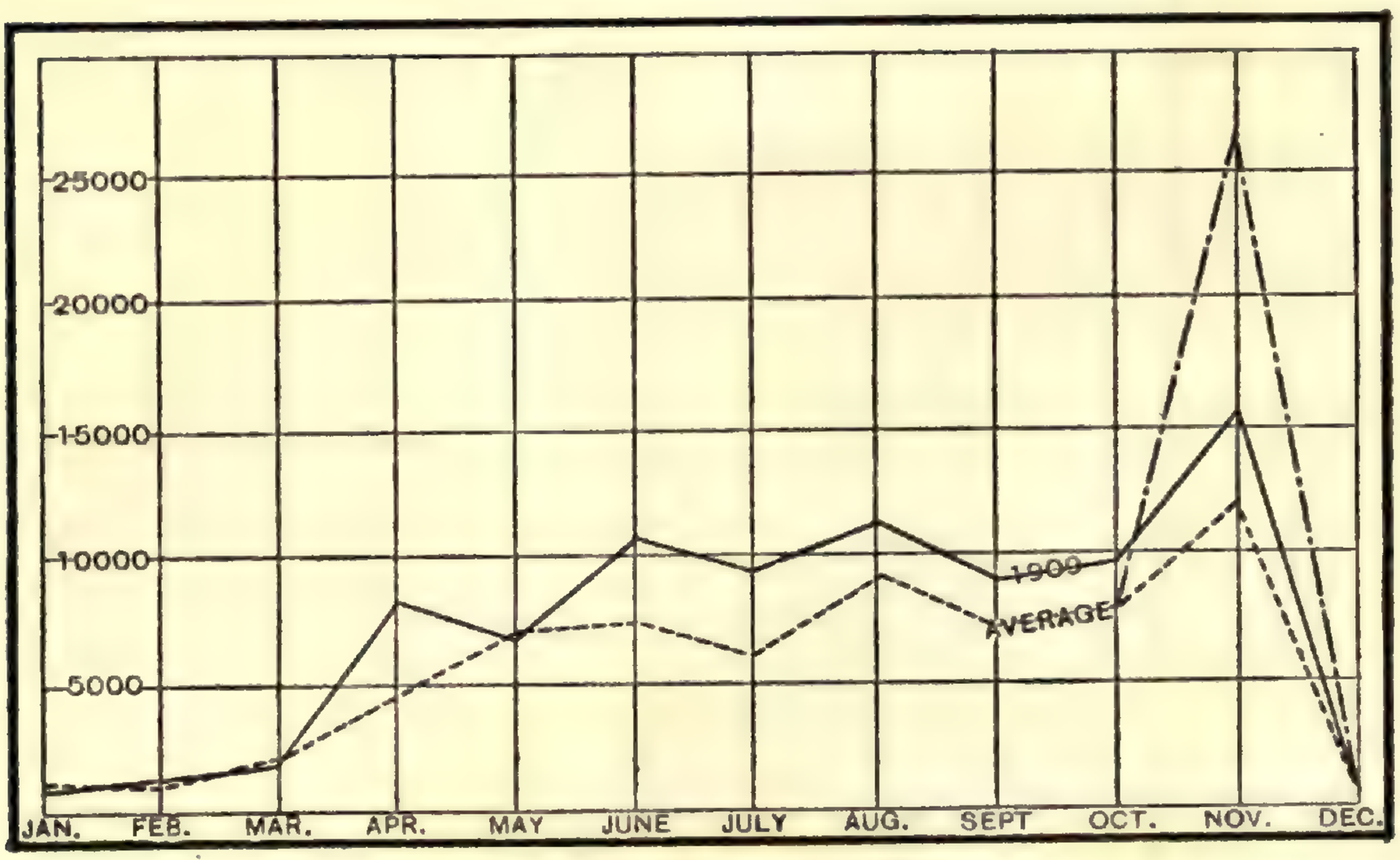
SUNDAY VISITORS, 1890 TO 1909.

with the average for previous records in January, February, March and May, but considerably exceeded this average in the other months except December. In November, however, they fell far below the average for the past few years, though the fact that over 16,000 were recorded justifies the special chrysanthemum display by which the larger part of these were attracted—the courtesy of Captain Robert McCulloch having again made possible the lighting of the tent so that it might be opened in the evening.

ON AN "OPEN SUNDAY."

The number of visitors for 1909 is 120,748, of whom 18,379 were recorded on the first Sunday afternoon in June and 17,572 on the first Sunday afternoon in September. The relation of these 35,951 Sunday visitors to those of other years, and to the total for this year (29.7%), is indicated in Diagram C. The seasonal distribution of the 84,797 week-day visitors, and its comparison with the average for earlier years, are graphically presented in Diagram D. The total recorded for 1909 has been exceeded (apart from the Exposi-

DIAGRAM D.



WEEK-DAY VISITORS, 1909.

tion year) only in 1907, when the unusually large number of persons attracted by the chrysanthemums brought the number for the year to 135,497.

THE HERBARIUM.

Rather few large purchases of specimens have been made, but the herbarium has been enlarged by the customary current collections offered for sale. Advantage has been taken of the lessened purchases to have the unincorporated accumulations of recent years mounted; and relatively little unmounted material now remains. Eight hundred duplicate

specimens, valued at $40.00, were distributed to correspondents during the year.

The additions to the mounted collection in 1909 number 36,959 sheets of specimens, of which 6,344, valued (unmounted) at $317.20, were presented; 2,332, valued at $116.60, were collected by employees; and 28,283 were purchased, the Secretary's books showing an expenditure of $1,508.69 for specimens and material during the year.

The herbarium, so far as now mounted, consists of:

The Engelmann Herbarium (all groups)		97,859 specimens.	
The General Herbarium:—			
Higher plants.			
The J. J. Bernhardi Herbarium .	62,507		
The Henry Eggert Herbarium[2] .	26,703		
The J. H. Redfield Herbarium .	16,447		
The Sturtevant and Smith Herbarium	7,446		
The Gustav Jermy Herbarium .	5,118		
The A. W. Chapman Herbarium[2]	3,536		
The Julien Reverchon Herbarium[2]	17,210		
The Nicholas Riehl Herbarium .	3,359		
Other specimens	362,099	504,425	"
Thallophytes.			
The J. J. Bernhardi Herbarium[2]	3,191		
The Gustav Jermy Herbarium .	1,659		
The S. M. Tracy Herbarium[2] .	4,334		
The Wm. Trelease Herbarium .	11,000		
Other specimens	33,357	53,541	"
Making a total of		655,825	"
Valued at			$98,373.75

Supplementing the herbarium, and the shelved or incorporated exsiccatae, which are here counted as a part of it,[4] the Garden possesses specimens of economic plant-products, woods, seeds, etc., valued at $280.00, and 1,851 preparations for microscopic study, valued at $410.00, which have not been added to since their enumeration in my last report.[5]

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[2] So far as yet incorporated.

[3] This valuation at the rate of $15.00 per hundred mounted sheets.

[4] Rept. Mo. Bot. Gard. **16**: 21. [5] See Rept. Mo. Bot. Gard. **20**: 26.

The addition to the herbarium and library building reported last year has made it possible for the herbarium to be united under one roof again; and one floor of the new building has been equipped with steel cases, as nearly dust and insect tight as such cases can be made, the unit size adopted being a ground plan of 40¼×55¼ inches and a height of 90 inches. Advantage was taken of the necessary handling of specimens to rearrange them in the now accepted phylogenetic sequence.

THE LIBRARY.

The increase in the library during 1909 has been closely comparable with that for a number of years preceding; the more essential current publications have been procured, in addition to a number of works rounding out the library in the fields of bacteriology and phycology, though unfortunately means have not been found to add more than a very few of the earlier books which now and then appear in the market and are becoming more difficult of purchase and more costly with the passage of each year.

Additions number 883 books, valued at $1,886.45, 1,631 pamphlets, valued at $285.15, and 6 manuscripts, valued at $114.00, presented or received in exchange for Garden publications; and 485 books and 219 pamphlets bought, the expenditure for purchases and binding amounting to $2,727.80.

The card index has been increased by the addition of 45,533 cards, of which 35,301 were written by employees and the remainder purchased. As for several years past, in addition to indexing the plant illustrations of the library, the attendants have given special attention to references to germination and seedlings.

The serial publications now received number 1,464, of which 107 are bought and 1,357, issued by 970 institutions or publishers, are received in exchange for the Reports of the Garden. This number is twelve more than that reported last year. It is to be observed that to this exchange use of the Garden publications a large part of the yearly accessions to the library is directly attributable.

As now constituted, the library contains:

Pamphlets				36,201	
Books				25,453	
				61,654, valued at	$96,182.79
Manuscripts:					
Engelmann, Notes and Sketches	60,	valued at		$600.00	
Engelmann, Thesis	1	"	"	100.00	
Roetter, Sketches	1	"	"	100.00	
Shaw, Notes	5	"	"	100.00	
Sturtevant, Index Rerum	11	"	"	110.00	
Price, Bird and Insect Sketches	2	"	"	250.00	
Leconte, Insect Sketches	8	"	"	225.00	
Bay, Bibliotheca Riviniana	1	"	"	25.00	
Lindheimer, Miscellaneous	1	"	"	10.00	
Theses by Garden Pupils	10	"	"	10.00	
Theses by Graduate Students	8	"	"	8.00	
	108	"	"		1,538.00
Total	61,762	"	"		$ 97,720.79
Index cards	719,377	"	"		7,193.77
Total value of library					$104,914.56

Though it has not been possible to provide for the entire library under one roof, as was done for the herbarium, the addition to the library building has made it possible to bring the most-used classes of books once more together, those left in the old Museum building, though less quickly accessible, being safely housed. Two floors of the new building have been equipped with steel cases corresponding in style, length, and height with those used in the herbarium, so that books referring to a given group of plants are appropriately shelved near the specimens; both the herbarium and library are capable of rearrangements and replacements at will, as they increase and are fitted into new quarters from time to time. To correspond with the changed sequence of families in the herbarium, the portion of the library that deals directly with taxonomy has been readjusted so as to follow the same classification.

THE GRADUATE LECTURE ROOM.

RESEARCH AND THE USE OF FACILITIES.

The long-established policy of making the facilities of the garden available to investigators has been taken advantage of by a number of visiting botanists. During the year 19,771 herbarium specimens have also been loaned to 26 investigators who were unable to visit Saint Louis or who could not complete their use of material at the Garden; and 360 books or pamphlets have been loaned similarly to 73 persons.

As in previous years, such part of the time of capable employees as could be given to original study has been so used, and a number of papers have been published embodying its results.

THE HENRY SHAW SCHOOL OF BOTANY.

One of the most cherished purposes of the founder of the Garden was the provision of means and appliances for instruction in botany—referred to by him not merely as a specific science but "in its application to horticulture, arboriculture, medicine and the arts,"[6] and with special mention of "vegetable physiology, the diseases of plants, the study of the forms of vegetable life and of animal life injurious to vegetation, experimental investigations in horticulture, arboriculture, etc."[7] Inaugurated before Mr. Shaw's death by the endowment of a School of Botany as a special department of Washington University[8] with provision for the closest affiliation between this department and the Garden[9] and with authorization for the Trustees of the latter "to allot, if they think it expedient, from time to time, any of the income not needed for the development and maintenance of the said Garden to the augmentation to the means and appliances of instruction,"[10] the realization of this purpose has necessarily rested, until the present year, upon the single professorship established in 1885.

Though still burdened by the necessary cost of holding the large tracts of unimproved and unproductive real estate

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[6] Rept. Mo. Bot. Gard. 1:36. [7] *l. c.* 1:37. [8] *l. c.* 1:56–59.
[9] *l. c.* 1:36–37. [10] *l. c.* 1:37.

in which a large part of the endowment of the Garden consists, the Trustees, after careful consideration, have decided that the time has now arrived for availing themselves of the authorization given for augmenting means and appliances for instruction, and as a first step toward this they have built and equipped laboratories suited to graduate work in certain lines of botany, and have established the post of plant physiologist at the Garden. To this post, Dr. George T. Moore has been called by the Trustees, and he has been elected by the Directors of Washington University to a newly established professorship of plant physiology and applied botany in the School of Botany. In connection with this professorship, two research fellowships in botany have been established which are this year held by Mr. E. G. Arzberger and Miss Ada Hayden.

The recently published Catalogue of the University enumerates the following regular courses now offered by the School of Botany in addition to special work suited to the individual needs of advanced students who, when possible, are given such work in preference to less elastic courses:

COURSES OF INSTRUCTION. — *A. Primarily for undergraduates:* 1, 2. General Botany.—2a, Field Ecology. — 3, 4. Plant Physiology. — 5. Bacteriological Methods. — 6. Histological Methods. — *B. For undergraduates and graduates:* 7. Bacteriology. — 9. Morphology and Taxonomy of Fungi. — 10. Morphology and Taxonomy of Algae. — 11. Morphology and Taxonomy of Bryophytes. — 12. Morphology and Taxonomy of Pteridophytes. — 13. Morphology and Taxonomy of Spermatophytes. — 16. Plant Ecology. — 18. Advanced Physiology. — 19. Water and Sewage Purification.

RESEARCH UNDER SUPERVISION. — Taxonomy. — Applied Mycology. — Physiology.

INDEPENDENT RESEARCH, in any field for which the necessary equipment is available, is open to persons qualified by training and experience to carry it on without instruction. To such advanced investigators the utmost freedom is allowed, and all the facilities of the School of Botany and the Botanical Garden are placed at their disposal free of expense, unless their work is done in candidacy for a degree, in which case the prescribed fees are chargeable.

The electives offered to undergraduates and jointly to undergraduate and graduate students, are intended in a general way to indicate the breadth of botanical preparation ex-

pected of successful candidates for the Master's degree in botany; while the research courses indicate the field in which work leading to the Doctor's degree may now be undertaken.

Undergraduate work, in the year just closed, has differed little from that of several seasons past. From the opening of the current college year, Mr. C. D. Learn, as Teaching Fellow in Botany, has continued the assistant's work performed last year by Mr. Nehrling. The undergraduate enrollment for the first term of 1909-10 was: Biology, eight, Botany 1, twenty; Botany 3, seven; Botany 5, five—a total of forty students, of whom the eight first noted give equal time to botany and zoology and the others take one full botanical course each.

On the completion of the new building at the Garden, one three-story pavilion, about fifty feet square, was reserved for use in connection with the graduate needs of the School of Botany and independent research, and an excellent equipment has been provided for work in bacteriology and other branches of mycology, phycology, and certain lines of plant physiology. In these directions research work, under the immediate direction of Professor Moore, is being carried out by four candidates for advanced degrees; while another applicant for the Master's degree is giving a part of his time to less advanced graduate study.

GARDEN PUPILS.

In March, Mr. Arno H. Nehrling and Mr. Henry Ochs, who had completed the prescribed course of study and passed an examination satisfactory to the Garden Committee, were given the customary certificates; and in June, Miss Herta A. Toeppen, who had also completed the required course and passed the requisite examination, was granted a similar certificate. On the results of competitive examination, duly announced, the scholarships released by Mr. Nehrling and Mr. Ochs were awarded to Mr. Clark Craig, of Rush Lake, Wisconsin, and Mr. Carl Haltenhoff, of Gotha, Florida. In June Mr. Raymond B. Wilcox, for family reasons, gave up the scholarship which he had held since 1907, and it was given to Mr. Homer E. Reed, of Louisiana, Missouri,

who had ranked highest among the unsuccessful competitors for the two scholarships awarded in the spring. At the end of December, the scholarship held by Mr. Jesse B. Tuggle was relinquished, and is still free.

No changes have been made during the year in the teaching staff or the prescribed course of study; but several of the Garden pupils have found time for additional work, of college grade, as special students in Washington University.

THE GARDEN STAFF.

Aside from a few changes among library assistants engaged temporarily in indexing and cataloguing, no changes are to be reported further than the addition of Professor Moore, already referred to. As in previous years, I can not too warmly commend the cheerful and efficient assistance that has been rendered by my associates, in caring for the important collections to which the Garden owes its chief value as an establishment for research, and I am especially indebted to Miss Hogan and Miss Brown for important contributions to the last Garden Report.

SPECIAL TESTAMENTARY PROVISIONS.

Three of the annual events provided for in the will of Mr. Shaw have taken place in 1909.

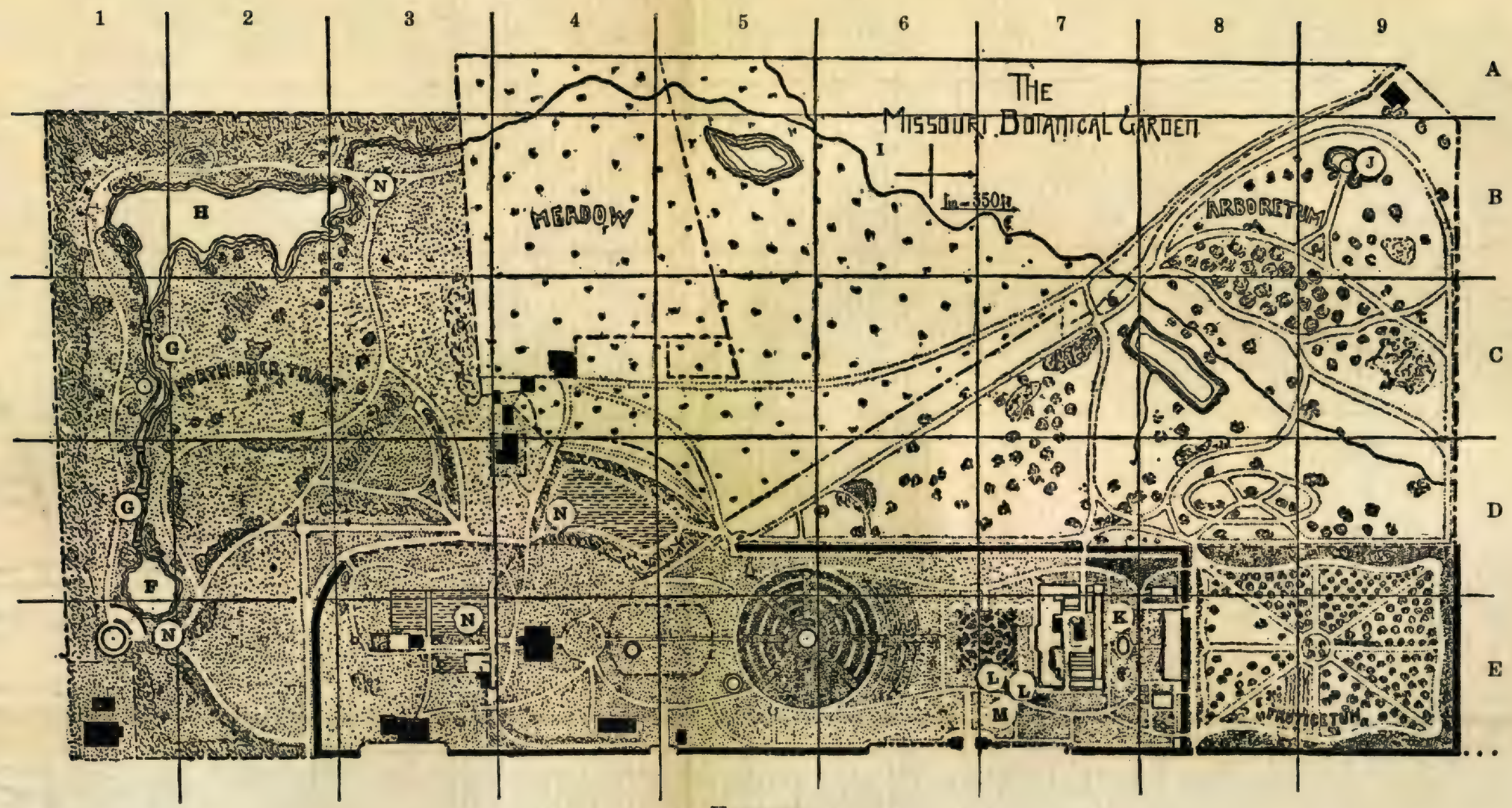
The flower sermon was preached in Christ Church Cathedral, St. Louis, on the morning of May twenty-third, by Rt. Reverend F. K. Brooke, Bishop of Oklahoma.

The sum set apart for floral premiums was once more entrusted to the St. Louis Horticultural Society, for use in connection with an exhibition held between November ninth and twelfth, but no award was made of the Shaw Medal.

The twentieth Gardeners' banquet, in the form of an open-air collation, was given in the grounds of the Director's residence at the Garden on July first; some 150 persons being present, of whom many were in attendance at the summer meeting of the State Board of Horticulture.

Very respectfully,

WILLIAM TRELEASE,
Director.



HABITATS.

F.	Fountain Pond.	(D 1).	L.	Nelumbium Pools.	(E 6–7).
	Typha Pool.	(D 1–2).	M.	Nymphaea Pool.	(E 6–7).
G.	Waterfall Stream.	(C–D 1).	N.	Earth Localities.	
H.	Lagoon.	(B 1–3).		Vegetable Garden.	(E 3).
I.	Arboretum Stream.	(A–D 3–9).		Mint Bed.	(D 4).
J.	Arboretum Pond.	(B 9).		Fountain Pond Beds.	(E 1).
K.	Crescent Pool.	(E 7–8).		Arboretum Stream.	(B 3).

Waterfalls all numbered westwards, from F. to H.

SCIENTIFIC PAPERS.

THE ALGAL FLORA OF THE MISSOURI BOTANICAL GARDEN.[1]

BY ADA HAYDEN.

INTRODUCTION.

While holding a research fellowship in the Henry Shaw School of Botany at the Missouri Botanical Garden an opportunity was afforded to investigate the algae found in the pools, ponds and streams of that place. The work was done under the direction of Professor G. T. Moore and the determination of the species in the systematic list, based on standard taxonomic works and exiccatae, has been corroborated by Professor Moore. The photographs of the habitats were made by Mr. Emil G. Arzberger and the species marked by an asterisk are taken from a manuscript list prepared by Dr. Henri Hus some years ago.

Any habitat study of plants involves ecological principles. In the present consideration of the garden algae none of the main habitat factors *i. e.*, light, heat, temperature and water vary from what may be considered typical for this region. While the water is artificially conducted to ponds and pools its source is the Mississippi and though passed through the purification process its chemical value as regards mineral content (U. S. Bull. Bur. Pl. Ind. No. 64) is not essentially different from that to be found in any chance location. It is seldom, however, that such a variety of types are found in such a limited area. This may be accounted for by the fact

[1] Abstracted from a thesis presented to the Faculty of Washington University, in candidacy for the degree of Master of Science, June, 1910.

that the artificial arrangement of pools, ponds and streams brings within a small space a varied number of natural conditions such as small bodies of quiet, shallow, constantly renewed water with or without outlet, swiftly and slowly running streams, etc.

So far as the sources of a fresh-water algal flora are concerned, there are present the possibilities of dissemination by (1) currents of air bearing spores through short distances; (2) transportation by birds, animals and insects. It has been observed in a study of the dissemination of *Lemna,* by Mr. C. H. Thompson, that *Belostoma americanum,* commonly found flying about electric light globes on the street, carried *Lemna* attached to its body. This insect stays in the water during the day and flies about at night. If this is true of *Lemna* it might easily be true of algae which grow in the same habitats. (3) Introduced plants in ponds and pools, many of which are of tropical origin, thus having been derived from widely differing habitats. There is but slight possibility of dissemination through the agency of water currents directly except within the garden itself, for only two small streams enter it, and the boundaries due to street or grading are higher than the surrounding territory. Of the two small streams, which enter, the one from the south drains the grassy, sparsely wooded portion of Tower Grove Park and during the period under observation has been quite free from algal growth large enough to be noted without microscopic inspection. The stream which comes from an ordinary city block on the west contains few or no algae and is often dry. It is evident from the chart that the main bodies of water within the garden are connected and it is to be noted that the city water at its entrance is free from all vegetation.

Having considered these deviations from the probable typical environmental characters and those characters bearing on the source of the algal flora, it is evident that this large number of forms present, offers a good opportunity for consideration of their relative grouping and adaptation to habitat conditions which in range and variety express in miniature the probabilities of a much larger area.

HABITATS.

The habitats observed in this study are indicated by letters on the phyto-geographic map. For purposes of discussion they may be resolved into the following types:

I. **Moist earth.**

II. **Water.**

Running.

a. **Waterfalls.**

b. **Rocky stream beds, *e. g.*, Arboretum stream.**

c. **Smooth stream beds, *e. g.*, Arboretum stream in part. Waterfall stream in part.**

Quiet.

a. **Ponds or pools with no outlet, *e. g.*, Crescent pool. Arboretum pool and Nelumbium pools.**

b. **Ponds or pools with outlet, *e. g.*, Fountain pond lagoon.**

Having viewed the general problem of habitat characters it is in order to consider the particular conditions in the locations studied which for convenience may be designated as Fountain pond, Waterfall stream, Lagoon, Arboretum stream, Arboretum pond, Nelumbium pools, Nymphaea pool, Crescent pool. Their location in the garden may be seen in the phyto-geographic map. The first four in the series are connected in the order named and as may be seen lie respectively in the lowest part of the tract between rolling elevations which drain into them. The surrounding territory is grassy with the exception of the Arboretum stream where the herbage is sparse due to the shade and more or less of loose soil. The fact that these bodies of water are connected makes quite probable the dissemination to succeeding connected bodies of water of anything which grows in the first of the series.

Some of the more obvious habitat characters in conformation to which plants arrange themselves in groups may be mentioned. The amount of moisture in the earth depends on precipitation, air currents and heat, of which the two latter affect evaporation from the earth; also level of water table and type of soil whose greater or less porosity affects the water holding and retaining power.

In water habitats are encountered such factors as depth, motion, rapidity of running water; attachment surfaces, *i. e.*, vertical faces of rocks subject to direct force of water; concave or protected surfaces under waterfalls where the atmosphere may be saturated and surfaces moist though plants are not submerged; soft, smooth, muddy floors of streams from which thalli may be easily swept by variation of currents depending on variation in volume of water which fluctuates periodically with rains; rocky, brick or cinder covered beds; the better aeration of water rapidly running over rocky beds; turbidity of water; presence or absence of sewage or decaying vegetation; wave beaten rock surfaces subject to variable water level; stagnant pools with no outlet; and little aeration with tendency to accumulate vegetation.

Stability of Habitat Characters.

These particular habitat characters are naturally affected by the more general meteorological conditions and granting that the physical characters of a habitat are fairly constant, the percentage of plants that might be able to live under those physical conditions at that time may become necessarily diminished in that some may become so prolific as to monopolize so large a proportion of light, moisture, space or nutriment, *i. e.*, life necessities, as to crowd weaker ones out. These stronger dominant plants, however, may serve as a protection to another type which might not have found the original conditions favorable for existence, hence the equilibrium is subject to continuous variation. It is evident that physiological, physical and biotic factors in the ecological problem are closely related. While physiological factors are of vital importance in the study of associations it will hardly be possible to give them specific consideration though an attempt will be made to note as far as possible the influence of physical and biotic factors.

Analysis of Habitats.

The Fountain pond is at an average 100 ft. in diam. and 4-5 ft. deep in the center, gradually sloping to the edge. The

water (city water derived from the Mississippi) enters at two points, the fountain in the center of the pond and a pipe at the south side. The level is fairly constant except in rainy weather, when it is variable. The water as it enters the pond maintains a fairly constant temperature, varying slowly through the year, the temperature being practically the same as that of the earth at four feet. It is covered in the winter with a coat of ice for about two months. The algae here group themselves into free swimming, those attached to water plants such as *Nymphaea* and *Potamogeton* (stationary) or attached to *Azolla* (floating). The free floating groups are swept across the surface of the pond by winds and in overflows after rains are swept down the stream.

Of the algae listed for this habitat, *Bulbochaete,* the *Spirogyras, Mougeotia, Palmodictyon, Cladophora,* and *Oedogonium* were at some stage attached. The others were floating or free swimming. *Pleodorina* was always found in warm, shallow water near the edge of the pond in a tangle of filamentous algae and small water plants as was generally true of the Volvocaceae in this pond. This piece of water contains several Spirogyras, only three of which have been determined, since conjugation had not taken place. All the filamentous forms are more or less closely associated in masses, *Spirogyra* and *Oedogonium* being predominant.

Typha angustifolia pool. This pool at the edge of the Fountain pond is about 4×6 ft. in size and seldom contains more than five inches of water, usually about two inches, and is often muddy without standing water. It contains a thick growth of *Typha angustifolia.* Observations were not taken here until March when an interesting association was found.

Mougeotia scalaris, Spirogyra tenuissima and *Gonatonema* sp. formed a closely interwoven mass in the spaces of which were several species of *Cosmarium, Closterium* and one of *Micrasterias.*

Waterfall stream. This stream connecting the Fountain pond and Lagoon contains fourteen waterfalls, all of which have limestone surfaces with the exception of X and XII, whose surfaces are cement covered.

Cladophora sp., *Pleurococcus vulgaris,* and *Stigeoclonium tenue* are the only forms found on the rocks themselves. *Pleurococcus* grew on all the rocks presenting a saturated surface, except the cement falls and *Cladophora* grew below the water-level of the stream at the base of the waterfalls X, XI, and XIII. Some of this *Cladophora* was the same species as that which grew on the rocks of the Lagoon. *Stigeoclonium* was present on all the falls in the parts where the water ran rapidly or struck breaking into spray; especially where it fell perpendicularly on a horizontal surface, these being the points of greatest aeration. A few small plants sparsely scattered were found on the perpendicular surfaces of the two cement waterfalls X and XII over which the water ran slowly in an unbroken stream. Whether the undesirability indicated by the absence of vegetation here was due to the cement or to the flow of the water is difficult to say. So far as water is concerned conditions were similar to those where *Cladophora* and *Pleurococcus* grew on the other falls but none appeared here. *Stigeoclonium tenue* grew abundantly on waterfall I all winter, but by the 16th of May was quite degenerate, the fall becoming covered with small leeches. *S. tenue* was noted on all of the falls but X, whose face was shaded by cat-tails and IX, over which the water ran sluggishly with little fall. All the other forms found in the intervals of the stream between the falls have been noted in the Fountain pond with the exception of *Hydrodictyon,* which probably originated there. It grew in a tangle of filamentous forms among the stems of cat-tails.

The Lagoon is a long, irregular body of water 130×540 ft. and approximately 12 ft. max. depth. It is deepest at the west side and slopes gradually toward the east. This larger body of water yields slowly to temperature changes, freezing later than the upper pond. On cold mornings when the small pond had a fringe or thin coating of ice the lagoon had none. The greater size makes the force of the wind of more importance here as waves starting from one side on reaching the other beat the floating fronds against the earth. *Oedogonium* is abundant on the shallow east side of the lagoon. The

vegetative state which lived through the winter in the lagoon compared with that of the upper pond and stream was found to be badly beaten by the wave motion. The pond on the south and west side becomes abruptly deep. The prevailing winds during the growing season are in a southerly direction. These combined factors seem to make the shallow east and north side a more valuable field for algal forms which are practically absent from the west side except at the northwest rocky dam which separates the lagoon from the overflow stream. Here on the rocks is a group which must adapt itself to the wave motion and rise and fall of the water level which is variable within a range of 6-8 inches.

The lagoon, probably due to the fact that it is a larger body of water with more variability of habitat shows less connection between groups. On the deep south and west shores only a few strands of *Oedogonium* were noted. On the shallow east side, abundant *Oedogonium,* apparently the same species as that in the Fountain pond and some *Spirogyra* were present. On the mud at the north end, in March for a brief period, a thick film of *Chlamydomonas* was seen. The rock of the dam at the west was the most favorable situation. There a slender *Cladophora* sp. grew all the year. *Spirogyra, Phormidium, Oscillatorias,* Desmids, Diatoms and *Tetraspora* were prominent during parts of the year. The most conspicuous association and the only well-marked one was that of *Anabaena Flos-aquae, Clathrocystis aeruginosa* var. *major,* and *Oscillatoria Agardhii* floating on the water often mixed with quantities of soot when the wind blew the smoke in that direction. (See *Anabaena* description.) Conjugatae and Cyanophyceae are predominant.

Arboretum stream. The water from the lagoon after it enters the pasture to the northwest contains only some fragments of *Spirogyra* or *Oedogonium* until it enters the Arboretum. Near the center of the Arboretum the incline becomes more abrupt than above, the bed is here narrower and rough with bricks and stones. Here *Cladophora* flourishes. Toward the east side of the Arboretum the bed widens and the slope is slight so that the current is sluggish. In this

region a sewage pipe enters. Here *Oscillatoria* is dominant. The floor of this stream is smooth and muddy. The surface of the water is never frozen in winter. It varies in volume with precipitation and becomes very turbid at these times.

Cladophora canalicularis during the winter was covered with diatoms which as the spring advanced diminished until by the first of May they had disappeared. The plants looked ragged and unhealthy at this time, but soon began to branch and regain their bright green color. On the stones near *Cladophora, Stigeoclonium glomerata* suddenly appeared in the spring and within a month disappeared. In the east end of the stream where the water was sluggish the Blue Greens were the principal forms. On the mud at the edge of the water in this region, a small form of *Vaucheria* grew,—a different species from the one in the Arboretum pond. Here the dominant forms were present during the whole period of study.

Arboretum pond. This is a small crescent shaped body of water from a few inches to two feet deep. It has no outlet so that the water is stagnant. It contains much decaying as well as living vegetation, consisting of *Typha, Juncus, Iris, Acorus* and *Nymphaea,* which makes conditions favorable for a rich growth in algal forms. This pond freezes earlier and remains frozen longer than the Fountain pond.

This body of water has more characteristic forms than any other. In the west wing planted with *Iris* is about ½ in. of water. Here *Vaucheria* forms a thick mat with several Closteriums (see list) *Oscillatorias* and a *Lyngbya* scattered among its filaments. In the center and east wing in deeper water the other algae were attached to the stems of *Acorus* or *Nymphaea* or floating in a tangle of decaying vegetation. *Oedogonium* was very rare here, though common in the habitats which had *Cladophora. Tetraspora* was first attached then floating over the whole water surface. Scenedesmaceae were practically absent. Chaetophoraceae are predominant. This is the only location in which Ulothricaceae were found.

Nelumbium pools. These are two cement basins with no outlet. The water is 2–3 in. deep. They are covered in win-

ter by mulching, freeze early and remain frozen late. The *Nelumbium speciosum* which grows in them is a tropical plant native to Eur-Asia.

There are two well marked seasonal groups here. None of the fall (Oct.-Dec. incl.) were observed in the spring (Mar.-May incl.) and vice versa with the exception of the diatoms which have been found in all the stations in greater or less abundance. The four species which were particularly characteristic in the fall are *Anabaena Flos-aquae, Nostoc, Spirogyra setiformis* and *Pithophora Mooreana* (see syst. list) a new species. These genera by history are well adapted to tropical life. *Oedogonium* though represented in some degree in most of the stations was rare here. The spring group is free swimming, free floating including Volvocaceae, Flagellatae, Scenedesmaceae,—*Chlamydomonas gloeocystiformis, Euglena proxima* and *Gonium pectorale* being very abundant for a short time. This is the only location in the garden in which *Spirogyra setiformis* and *Pithophora Mooreana* were found. This with the fact that the *Nelumbium speciosum* is a tropical plant is of interest here. The genera *Scenedesmus* and *Phacus* are the best represented of the spring group with regard to species though the relative representation of each species is not great.

Nymphaea pool is a large cement basin 15×30 ft., situated between the two Nelumbium pools. The description of the Nelumbium pools applies to this one except that the water is from 6 in. to 1 ft. in depth. Here are found *Nymphaea Marliacea* var. *chromatella, N. Marliacea* var. *carnea, N. Gladstoniana* and *N. Robinsoniana.*

The algae in this pond were in the fall casually observed, not studied by weekly microscopic examinations, as those from the other locations. It was noted, however, that *Spirogyra setiformis* and *Pithophora Mooreana* were not present in the Nymphaea pool though the Nelumbium basins were but 4 ft. away. The most conspicuous thing here was a very abundant growth of *Spirogyra dubia* which was first attached to bricks in the pool becoming free as it rapidly developed. The filaments simultaneously went into conjugating state April

15, after which it sank to the floor of the pool. The free floating or swimming forms of which the other representatives consist seem scattered before this time, but when small masses of the conjugating *Spirogyra* were examined spaces between the filaments were found to be very abundantly filled with these small algae. Here Conjugatae, Volvocaceae, Scenedesmaceae and Flagellatae were represented. During the early part of May just after the *Spirogyra* had disappeared a large number of tadpoles made their appearance in the pool and the remaining small algal forms rapidly approached the vanishing point, the more abundant ones becoming rare.

Crescent pool. This is a small pool of the shape of its name with earth sides and floor and has no outlet. It contains *Acorus, Nymphaea,* several species, and *Nelumbium.* Observations were here taken during the spring, in the early part of which one form was found in this place only, i. e., *Stigeoclonium glomerata.* This pool contains more Oscillatorias than the other pools. In the latter part of April it was cleaned preliminary to the spring planting and the only forms which endured were the group of Oscillatorias.

Earth habitats. Those in which algae have been studied are: 1. Vegetable garden, *Botrydium Wallrothii* and *Protosiphon;* 2. The mint beds, *Botrydium Wallrothii;* 3. Flower beds near Fountain pond, *Protosiphon botrioides, Chlorococcum humicola, Oscillatoria animalis* and *Stichococcus subtilis;* 4. Edge of Arboretum stream, *Vaucheria* sp.

The soil of the first three locations is loess, moderately moist ordinarily, occasionally becoming quite dry on the surface. The fourth location is usually saturated, simply mud.

SYSTEMATIC ENUMERATION.

Schizophyta.

SCHIZOMYCETES.

BEGGIATOACEAE.

Beggiatoa alba (Vauch.) Trev. Stagnant water containing sewage or decaying vegetation. Arboretum stream.

SCHIZOPHYCEAE.

CHROOCOCCACEAE.

Chroococcus limneticus var. *sub-salsus* Lemm. Appeared end of May in Nelumbium pool.

Coelosphaerium Kützingianum Näg. In quiet water with larger algae. Fountain pond.

Coelosphaerium confertum W. and G. S. West. Nelumbium pools. Nymphea pond.

Clathrocystis aeruginosa var. *major* Wittr. This species was noted near the end of September, associated with *Oscillatoria Agardhii* and *Anabaena Flos-aquae* forming a conspicuous green scum on the surface of the water of the Lagoon. *Clathrocystis* was seen as late as the end of November, but *Anabaena* and *Oscillatoria* had disappeared by that time. This association has been observed by Möbius in the Botanical Garden at Frankfort with the substitution of *C. aeruginosa* for *C. aeruginosa* var. *major*.

Merismopedia tenuissima Lemm. Nymphaea pool March to May.

Merismopedia elegans A. Braun. Waterfall stream. Lagoon. Sept. to Nov.

Merismopedia convoluta Bréb. Arboretum stream. Fountain pond. Sept., Oct., Nov., June.

OSCILLATORIACEAE.

Oscillatoria Agardhii Gomont. Lagoon on surface of water associated with *Clathrocystis aeruginosa* var. *major* and *Anabaena Flos-aquae*. End of Sept. to end of Oct. (See *Clathrocystis.*)

Oscillatoria animalis Agardh. Mingled with *Stigeoclonium tenue* on the perpendicular surface of the first waterfall at the outlet of the Fountain pond. April. Noted on earth near *Protosiphon* in May. (E_3)

Oscillatoria amphibia Ag. Arboretum pond among *Oedogonium*. Some trichomes were noted 3.2μ wide, which is slightly larger than the type measurements. March, April. Rather common.

Oscillatoria tenuis Ag. Crescent pool, forming a dark green stratum on the floor of the pond,—closely associated with *O. animalis* and *O. limosa.* Mar., Apr. Abundant.

Oscillatoria chalybea Mertens. Arboretum stream. Associated with *O. limosa.* Sept., May. Common.

Oscillatoria formosa Bory. Arboretum pond. Nelumbium pool. Apr., May. Rare.

Oscillatoria limosa Ag. Lagoon. Crescent pool. Arboretum stream. Earth. This species occurs in greater abundance and in more habitats than any of the others. At the lower end of the Arboretum stream it is found in the greatest quantity. In the upper part of the stream very little is present. The upper half of the stream flows rapidly over rocks, while the lower half, into which sewage enters, flows sluggishly. Here in the sluggish part of the stream *O. limosa* covers the floor in a thick stratum, which now and then breaks up into tufts and floats away or is forcibly all carried away by freshets, after which, in a short time, the floor of the stream is recovered. Sept. to May.

Oscillatoria splendida Greville. Crescent pool. Associated with *O. limosa* and *O. tenuis.* Common.

Oscillatoria limnetica Lemm. Arboretum stream. Rare.

Lyngbya Digueti Gomont. Attached to *Stigeoclonium tenue* on 1st Waterfall and to *Vaucheria* in Arboretum pond. Rare.

Lyngbya Lagerheimii (Möb.) Gomont.*

Microcoleus vaginatus (Vauch.) Gomont.*

Phormidium uncinatum (Ag.) Gomont. On rocks West Lagoon. Very abundant, forming a thick dark green stratum. Sept. to Dec., Apr., through May.

Spirulina major Kütz. West Lagoon, associated with *Oscillatoria limosa.* Sept. to Dec. Common.

NOSTOCACEAE.

Anabaena circinalis (Kütz.) Raben. Quiet water. Lagoon. Rare. Oct.

Anabaena Flos-aquae (Lyngb.) Bréb. Nelumbium pool. Abundant in Lagoon. (See *Clathrocystis.*) Sept. to Oct.

Nostoc sp. Nelumbium pools. Sept. to Nov. Common.
Cylindrospermum minutissimum Collins. Nelumbium pools. May and June. Common.

SCYTONEMACEAE.

Scytonema Hofmanni Agardh.*

RIVULARIACEAE.

Calothrix sp. Nymphaea pool on edge of cement basin. Common. May, June. Associated with *Stigeoclonium aestivale, Chroococcus* and *Hydrodictyon.*

Flagellata.

RHIZOMASTIGACEAE.

Mastigamoeba aspera Schultze.*

HYMENOMONADACEAE.

Synura uvella Ehrenb. Arboretum pool. Sept., Oct. Rare.

OCHROMONADACEAE.

Dinobryon sertularia Ehrenb. Arboretum pond. Rare. Oct.
Uroglena volvox Ehrbg.*

CRYPTOMONADINEAE.

Chilomonas sp.*

EUGLENACEAE.

Euglena acutissima Lemm. Nymphaea pool. Rare.
Euglena acus Ehrbg.*
Euglena oxyuris Schmarda.*
Euglena proxima Dangeard. Nymphaea pool. Arboretum stream. Abundant. April, May, June. This species was noted thickly covering the surface of the Nymphaea pool, with a green scum. Many of the individuals were in motion, though the scum consisted largely of those in resting state, more or less enveloped in mucus and globular in form. At intervals this scum appears on the mud at the edge of the Arboretum stream.

Euglena spirogyra Ehrbg. Arboretum pool. Large Nelumbium pool. Fission observed Apr. 12.

Euglena viridis Ehrbg.*

Phacus longicauda Dujardin. Arboretum pool. Rare. Apr., May.

Phacus pyrum (Ehrbg.) Stein. Garden stream. Nymphaea pool. Sept., Oct., Mar., Apr., May. Rare.

Phacus pleuronectes Nitzsch. Arboretum pool. Waterfall stream. Nelumbium pool. Fountain pond. Common. Sept., Oct., Mar. to May.

Phacus triqueter Ehrbg.*

ASTASIACEAE.

Astasia sp.*

Bacillariaceae.

Navicula sp.*

Gomphonema acuminatum Ehrbg.*

Gomphonema constrictum Ehrbg.*

Heterokontae.

Confervales

CONFERVACEAE.

Ophiocytium sp. Arboretum pond on *Microspora*. Rare. April.

BOTRYDIACEAE.

Botrydium Wallrothii Kütz. (E3) Cabbage patch. Earth. Associated with *Protosiphon botryoides* (Kütz.) Klebs. Nov.

Botrydium granulatum (L.) Greville. Mint beds on damp loess soil (D4). Abundant. In the first part of March on the damp soil of a flower bed (soil had not been stirred since the previous summer) was noted what proved to be *Botrydium* aplanospores in such abundance that the earth looked light green. No *Botrydium* plants were observed at this time. At the end of March in another bed in a different location were found *Botrydium* plants in abundance forming dark green masses among the more conspicuous brighter green

aplanospores. In location I. at this time the earth had dried out, the aplanospores had disappeared and no plants were observed in this place.

At the end of March most of the plants were producing aplanospores which immediately began to grow into plants. The aplanospores found earlier in the season on the earth were studied directly from the earth and in drop cultures, but showed no indication of developing into plants at that time. Usually, as soon as these aplanospores taken from the earth were placed in water, they began to produce zoospores. Others of these same aplanospores, when mature, apparently divided into another generation of aplanospores within their walls, which broke, setting the young ones free. They usually remained agglutinized in a spherical group for a time, then broke apart and grew to normal size. All of the young aplanospore cells contained clearly defined, sub-angular chromatophores, which lost their outline, becoming diffuse granular as the cells increased in size.

The aplanospores, then, may develop into plants, remain resting for a time, produce zoospores or divide into other aplanospores, depending on environmental conditions, water probably being the most important. The normal size of the aplanospores up to the time of maturity as indicated by sending out a rhizoid process or internal re-organization, is 16—38.4μ.

Chlorophyceae.

DESMIDIACEAE.

Closterium acerosum Ehrenb. Arboretum pond. Nymphaea pool. Nelumbium pools. Crescent pool. Mar., May. Common.

Closterium acerosum var. *elongatum* Bréb. Arboretum pond. Nymphaea pool. Nelumbium pools. Crescent pool. Mar., May. Common.

Closterium Lunula var. *intermedium* Gutw. Lagoon. Fountain pond. Arboretum pond. Sept., through Nov. Mar. to June. Rather common.

Closterium moniliferum Ehrenb. Typha angustifolia pool. Lagoon. Oct., May. Rare.

Closterium strigosum Bréb. Arboretum pond. Mar., May. Rather common.

Cosmarium Botrytis Menegh *

Cosmarium Broomei Thwaite.*

Cosmarium granatum Bréb. Nymphaea pool. Apr., May.

Cosmarium margaritiferum Menegh.*

Cosmarium Phaseolus var. *minor* Boldt. Nymphaea pool. Mar., May.

Micrasterias americana (Ehrenb.) Ralfs. Typha angustifolia pool, with filamentous algae. Rare. May.

Pleurotaenium Trabecula (Ehrenb.) Näg. West side of Lagoon. Rocks. Rather common.

Pleurotaenium Trabecula var. *granulata* West. West Lagoon. Rather common.

Penium margaritaceum (Ehrenb.) Bréb. Nymphaea pool. Rare. May.

Staurastrum sp.

ZYGNEMACEAE.

Spirogyra dubia Kütz. Nymphaea pool. Abundant. Conjugating Mar. 15.

Spirogyra Grevilleana (Hass.) Kütz. Fountain pond. Waterfall stream. Rather common. Sept. and through May. Conjugating early in Mar.

Spirogyra longata (Vauch.) Kütz. Waterfall stream. Fountain pond. September and through May. The material in the garden does not exactly conform to the type description. There is, however, observed a variation in the measurements given for the vegetative cells as described by Collins, who gives 20-36μ as diameter; Petit 25-30μ; De Toni, 24-30μ. There is also more or less variation in regard to the length of the cells in descriptions. Collins describes the spore as broadly ovoid; Wolle, twice as long as broad, though all state that the spore completely fills the diameter of the cell without swelling, which is true in this case, whatever may be the width of the filament. The measurements of the garden specimens are: veg. fil. 21.4-28.8μ; chromatophore up to 6.4μ with as many as 5 coils. The vegetative cells are up to 10 diameters long, usually less;

spores 21.4-28.8×48-80μ, up to 3 diameters in length, ellipsoid with rounded tips, conjugating the middle of May.

Spirogyra porticalis (Müller) Cleve. Fountain pond. Waterfall stream. Sept., through May. In an instance where 3 filaments lay parallel, the two outer filaments contained zygospores, the result of conjugation of aplanogametes from the central strand. In two cells of the central strand were two zygospores, the cells of which showed connection with cells of adjacent strands mentioned, indicative that the whole strands were not of one sex, but that the distinction of sex applies to the individual cell. Conjugation observed at end of March.

Spirogyra setiformis (Roth) Kütz. Nelumbium pools. Fairly abundant. Conjugating in November. This is the only species which conjugated in the fall (Dec.)

Spirogyra tenuissima (Hass.) Kütz. Arboretum pond. Associated with *Mougeotia scalaris* and *Gonatonema* sp. Sept. to June. Conjugating through May. Conjugation is both lateral and scalariform. The receptive cell swells before dissociation of the chromatophore. Chromatophore of male cell is usually dissociated and ready to pass out of its cell before the chromotaphore of the receptive cell has lost its spiral form. In some cases two normal appearing zygospores (aplanospores?) were found in one cell. These were somewhat smaller than the ordinary spores.

MESOCARPACEAE.

Gonatonema sp. Typha angustifolia pool. Abundant.

Mougeotia scalaris Hassall. Arboretum pond, closely associated with *Spirogyra, Microspora, Stichococcus.* Quiet waters. Conjugation observed Apr. 5. In one instance 2 gametes from one filament were conjugating with one from another adjacent filament to form a zygospore.

VOLVOCALES.

CHLAMYDOMONADACEAE.

Chlamydomonas gloeocystiformis Dill. First noted forming a thick, bright green coat on the mud of the north branch

of the Lagoon, later forming a green scum on the surface of the Nymphaea pool.

The cell remains motile long, finally becoming motionless, assuming a spherical form and lying imbedded in a gelatinous secretion. The first division in the formation of young zoospores is longitudinal, the next transverse. Dimensions, 9.6-17μ×6.4-11.2μ, while active; 6.4-12.8μ resting.

VOLVOCACEAE.

Eudorina elegans Ehrenb. Fountain pond. Nymphaea pool. Waterfall stream. Quiet water. Sept. to Dec. Colonies were breaking up the early part of Dec.

Gonium pectorale Müller. Nelumbium pools. Nymphaea pool. Arboretum pond. Producing auto-colonies Apr. 10.

Pandorina Morum (Müll.) Bory. Fountain pond. Nelumbium pools. Arboretum stream. Waterfall stream. Nymphaea pool. Sept., Dec., Mar., through June. Producing auto-colonies abundantly in April and March.

Pleodorina californica Shaw. Edge of Fountain pond. Shallow water. Colonies were observed in perfect state the last of September. During the first part of November they lost their vegetative cells and the number of colonies decreased. The colonies at this stage resembled *Eudorina*. Sept., Nov.

TETRASPORACEAE.

Tetraspora gelatinosa (Vauch.) Desvaux. Arboretum pond. Rocks, west Lagoon.

Ineffigiata neglecta W. and G. S. West. Fountain pond. Rare.

PROTOCOCCALES.

PROTOCOCCACEAE.

Chlorococcum infusionum (Schrank) Menegh. Lagoon.

PROTOSIPHONACEAE.

Protosiphon botryoides (Kütz.) Klebs. Cabbage patch (E3). Associated with *Botrydium Wallrothii* in Nov. in abundance. In March, scarce on a flower bed near Fountain pond. May, abundant. In loess soil.

SCENEDESMACEAE.

Actinastrum Hantzschii Lagerh. Nymphaea pool. Mar., June. Multiplication takes place by longitudinal division of cells. Observed Apr. 6. The size of the individual found here differs from that given by De Toni, Hansgirg, West and Chodat, *i. e.*, 3.6μ×10-24μ. The dimensions of those found in the Garden are 2.4-3.2μ×9-16μ. Colony 19.2-32μ diam.

Ankistrodesmus falcatus (Corda) Ralfs. Fountain pond. Waterfall stream. Nelumbium pools. Nymphaea pool. Abundant. Sept. to Dec., Mar. through June.

Ankistrodesmus falcatus var. *spiralis* (Turn.) West. Nymphaea pool. April-June. *Raphidium polymorphum* var. *contortum* (Thur.) Wolle, and *Ankistrodesmus contortus* Thur., according to the descriptions given in Wolle (Freshwater Algae of U. S. 198. pl. CLX., seem to be synonymous with West's *Ankistrodesmus falcatus* var. *spiralis*. Thuret's description antedates the others here mentioned.

Ankistrodesmus falcatus var. *mirabilis* West. Nelumbium pools. Waterfall stream. Nymphaea pool. Sept. to Dec., Apr., June.

Ankistrodesmus falcatus var. *tumidus* West. Nelumbium pools. Fountain pond. Waterfall stream. Nymphaea pool. Oct., May. Rare.

Coelastrum cubicum Näg. Fountain pond. Common. Sept. and Oct. Rare. The form found in the garden corresponds closely with Lemaire's figure and descriptions of *C. cornutum,* which Senn discusses and declares is not sufficiently distinguished from *C. cubicum* to exist as a species.

Coelastrum microsporum Näg. Fountain pond. Quiet water of Waterfall stream. Common. Sept. to Dec.

Dictyosphaerium Ehrenbergianum Näg. Nymphaea pool. Most abundant near the floor of the pond. Apr., May. Colonies observed here consist of 4-16 cells. Diam. of col. 16-41.6 μ;cells rather uniform in size, measuring about 3.2-6.4μ. The cup-shaped chromatophore has a red eye spot. This form corresponds closely in size to the *Dictyosphaerium*

which Bernard describes (1909 Algues Unicellulaires) from Singapore. Though smaller than the type as generally described, he considers this variable character not sufficient to establish a new species.

Kirchneriella lunaris (Kirchner) Möbius. Fountain pond. Sept. to Dec. Nymphaea pool. Mar., June. Rather common.

Kirchneriella obesa (West.) Schmidle. Nymphaea pool. Mar. to June. Producing auto-colonies in April.

Scenedesmus bijuga (Turp.) Wittr. Nelumbium ponds. Nymphaea pool. Fountain pond. Waterfall stream. Sept. to Dec., Mar., June. Common.

Scenedesmus obliquus (Turp.) Kütz. Nelumbium pools. Fountain pond. Waterfall stream. Nymphaea pool. Rather common. Sept. to Dec., Mar. to June.

Scenedesmus obliquus var. *dimorphus* (Turp.) Hansg. Fountain pond. Nymphaea pool. Nelumbium pool. Waterfall stream. Sept., Dec., Mar., June. Rather common.

Scenedesmus quadricauda (Turp.) Bréb. Fountain pond. Nelumbium pool. Waterfall stream. Nymphaea pool. Sept., Dec., Mar. to June. Common. Auto-colonies in Oct. and Apr.

Selenastrum gracile Reinsch. Nymphaea pool. Rare. Sept., Oct., May.

Tetraedron trigonum (Näg.) Hansg. Nymphaea pool. April, May. Rather rare.

Tetraedron trigonum var. **pentagonum** (Rab.) New combination. Nymphaea pool. Apr., May. Rather rare.

Wolle has described *Polyedrium trigonum* Näg.: cells somewhat compressed, 3-5 angled; angles obtuse-mucronate, sides more or less concave. Kirchner has suggested the following names for varieties (a) *typicum* Kirch.; (b) *minus* Reinsch; (c) *tetragonum* Rab.; (d) *pentagonum* Rab.; (e) *punctatum* Kirch.; (f) *bifurcatum* Wille. (See Wolle, Freshw. Alg. of U. S. 184. 1887; Kirchner, Kryptfl. von Schlesien, **2**: 104. 1898.)

Hansgirg, in his revision of this genus and reversion to Kützing's generic name has not made provision for the five-

angled mucronate-tipped form. De Toni in his summation of the varieties lists *T. trigonum* (Näg.) Hansg. and *T. caudatum* (Corda) Hansg. He does not include *Polyedrium trigonum* var. *pentagonum* Rab. or any equivalent.

Tetraedron caudatum (Corda) Hansg. Nymphaea pool. Rather rare. Apr., May.

HYDRODICTYACEAE.

Hydrodictyon reticulatum (L.) Lagerheim. Waterfall stream. Nelumbium pool. Apr., May, June. Rather common.

Pediastrum Boryanum (Turp.) Meneg. Fountain pond. Nymphaea pool. Common. Sept., Dec., March to June.

Pediastrum duplex Meyen. Fountain pond. Nelumbium pools. Nymphaea pool. Sept. to Dec., Mar. to June. Rare.

Pediastrum tetras (Ehrenbg.) Ralfs. Nelumbium pools. Nymphaea pool. Fountain pond. Sept., Mar., Apr., May. Rather common.

ULOTHRICACEAE.

Microspora stagnorum (Kütz.) Lagerheim. Arboretum pond. Associated with *Spirogyra* and *Stichococcus*. Akinetes produced in the early part of April. Abundant. Mar., Apr., May.

Stichococcus subtilis (Kütz.) Klercker. Earth near Fountain pond. (E1) Apr., May. Rather common.

OEDOGONIACEAE.

Oedogonium sp. Fountain pond. Lagoon Waterfall stream. Arboretum stream. Abundant. Sept. to June. Zoospores abundantly produced in Sept., Oct., Nov. They were enclosed in a delicate enveloping sac in which they came out of the cell and from which they escaped in a few minutes. Sexual reproduction beginning in June.

Bulbochaete sp. Fountain pond. Sept., Oct., Nov. Rare.

ULOTHRICALES.

CHAETOPHORACEAE.

Chaetophora elegans (Roth) Agardh. Attached to stems of *Juncus*, mixed with filamentous algae. Arboretum pond. Apr., May. Rare.

Draparnaldia plumosa (Vauch.) Agardh. Arboretum pool. Jan., Apr. Rather common. Produced zoospores in March.

Stigeoclonium aestivale (Hazen) Collins. Arboretum pond. Growing on stems of *Nymphaea* and *Acorus*. Nymphaea pool on edge of cement basin.

Stigeoclonium lubricum (Dillw.) Kütz. Rocks in upper part of Garden stream, running water. This species was observed in the Garden stream in March only. It appeared abruptly, grew to maturity rapidly and disappeared as suddenly as it came. Though collections were made weekly, it was difficult to determine the exact time of its disappearance. It was noted by the 1st of April.

Stigeoclonium stagnatile (Hazen) Collins. Fountain pond. Floating, associated with *Spirogyra* and *Mougeotia*. Oct.

Stigeoclonium glomeratum (Hazen) Collins. Crescent pool. Abundant during March and early part of April. Pond was cleaned and it did not reappear. Cells of main branches were on an average 3–4 times the diameter of the cell. Long cells were rather exceptional, though they were noted up to 8 diameters in length. Great variation in branching was noted, some branches having few fascicles, while others showed a marked fasciculate tendency. The thalli in mass were very gelatinous.

Stigeoclonium tenue (Ag.) Kütz. Waterfall stream on face of waterfalls. The form growing in the garden is setiferous. It was found growing abundantly on the perpendicular surface of the first waterfall in Sept., where it grew with little variation in quantity through the winter and spring. Water ran over the fall all winter, though under a frozen surface coating of ice. It was observed in small patches in the autumn on several of the other falls, which presented a perpendicular or slanting surface, washed by the water. During the winter water flowed not over, but under the other falls, the surface of which dried out. The second waterfall presented a striking bright green patch on the horizontal surface of a shelving rock which the water struck as it fell

perpendicularly from a shelf above. Young *S. tenue* began to grow in the latter part of March. Zoospores were produced abundantly in the fall.

Microthamnion Kützingianum Näg. Crescent pool with *Stigeoclonium glomeratum.* Rare. April.

Palmodictyon viride Kütz. Fountain pond among filamentous algae and partially decaying aquatic plants. The cells of the thalli here were 6.4-9.6μ in diam., rarely 12.8μ. Rare. Oct., Nov., May, June.

Pleurococcus vulgaris Menegh., not Näg. On the saturated surface of rocks over which water seeps. Waterfall stream. Common. Sept., June.

SIPHONOCLADIALES.

CLADOPHORACEAE.

Cladophora canalicularis (Roth) Kütz. Attached to rocks in the bed of the rapidly running Arboretum stream. This *Cladophora* covered the rocks with a thick coat of cool green color during the fall, first observed in Oct., which became grayish, yellowish and dingy during the winter, when it became covered with a thick coat of diatoms. The diatoms began to diminish in number near the first of April, leaving the *Cladophora* looking ragged, limp and unhealthy. Some fresh new branches were then sent out from the old weather-beaten thalli, and young plants also began to grow. Some of the cells were filled with dense protoplasm resembling the prolific cells of *Pithophora.*

Pithophora Mooreana Collins MS.[2]

This *Pithophora* was first observed in the garden by Dr. Moore in Nelumbium pool. The description by Mr. Collins is not yet published. The plants were growing in Sept. and Oct. in the small Nelumbium pools. In Oct. spores were very rarely seen. After the first frost spores were formed

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[2] Closely related to *P. Summatrana* but differing in dimensions and in the grouping of spores, which are borne in groups of one, two or three in *P. Mooreana,* the spores of *P. Summatrana* being borne singly.

sparingly. Some *Pithophora* kept in a glass jar in water from the pool was observed in Jan. to have abundant spores. The plants at this time broke up easily. The akinetes were arranged 1, 2, and not uncommonly 3 in a group. Spores are usually borne at the upper end of the cell, terminal or intercalary. In a few instances swellings globular in form just below the akinete at the top of the cell, were noted. This swelling was preliminary usually to the formation of a spore, but in some cases there appeared to be not enough of protoplasm to form a second spore in the cell, while in other cases twin spores were developed in a cell. Twin spores were usually observed in the branches, not in the main stem. Akinetes of the main stem are cylindrical (square to rectangular in outline) little, if any, swelled. Akinetes of the branches were cask shaped. In twin groups the cells were either similar in shape or one cylindrical and one cask shaped. The branching is one ranked. In only two cases were rhizoid-like structures observed. The measurements are:

Akinetes.		Max.	Av.	Min.
	L.	380μ	114μ	95μ
	W.	114	95	57
Side Branches		665—	114μ	
Main Stem		114—	142.5μ	

Pithophora Oedogonia (Mont.) Wittr. Fountain pond. Oct., June. Akinetes produced in June. Rather rare.

Rhizoclonium hieroglyphicum (Ag.) Kütz.*

SIPHONALES.

VAUCHERIACEAE.

Vaucheria sp. Mar. to June. Submerged or terrestrial. West Arboretum pond and mud of Arboretum stream.

FOUNTAIN POND.

LAGOON.

ALGAL HABITATS.

FIRST AND SECOND WATERFALLS.

TWELFTH, THIRTEENTH AND FOURTEENTH WATERFALLS.

WEST ARBORETUM STREAM.

CENTRAL ARBORETUM STREAM

ALGAL HABITATS.

ARBORETUM POND—EAST SIDE.

ARBORETUM POND—NORTHWEST SIDE.

CRESCENT POOL.

NYMPHAEA AND NELUMBIUM POOLS.

ALGAL HABITATS.

PERIODICITY IN SPIROGYRA, WITH SPECIAL REFERENCE TO THE WORK OF BENECKE.*

BY C. H. DANFORTH.

The fact that there is a certain periodicity in the appearance of reproductive phases of many algae is a matter of common observation, and several attempts have been made to determine more or less definitely the factors controlling reproduction in these forms. In work of this kind *Spirogyra* has perhaps been the most frequent subject for experimentation and observation, but the work of Williams ('05) and Hoyt ('07) on *Dictyota* has yielded interesting, if somewhat puzzling results. The former of these authors was able to demonstrate a very constant and clearly marked periodicity in *Dictyota dichotoma* which he found to liberate the eggs and sperm at a definite time following the highest spring tide of each cycle. An analysis of the conditions that vary concomitantly with the tide, and the fluctuations in the tide and time of sexual maturity of *Dictyota* on the various parts of the British coast led the author to believe that the observed periodicity in the reproduction of this plant is controlled by the amount of light received, which in turn is regulated by the tides. Nevertheless plants kept in an aquarium were found to show the same periodicity the following spring despite the fact that they had been for a long time removed from all periodic fluctuations except those of day and night. Williams therefore concluded that the periodicity manifested by *Dictyota dichotoma* is an inherited characteristic which in nature comes to synchronize with the tidal periods.

Hoyt studied the same species on this side of the Atlantic

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* The work recorded in this paper was done in the graduate laboratories of the Henry Shaw School of Botany at the Missouri Botanical Garden and the author's acknowledgments are due Professor George T. Moore for suggestions and assistance.

and while his data as to the time of liberating the sexual products do not exactly coincide with those of Williams, the general features of the cycle are the same and he too was able to demonstrate a periodicity in aquarium plants that agreed closely with that shown under natural conditions, even though the gametangia were born on parts that had developed entirely in the laboratory and had consequently never been subjected to the usual periodic variations in the environment. He also obtained some evidence to the effect that plants growing in seas where there are almost no tides likewise show a similar periodicity but, as the author remarks, the evidence on this point is perhaps not sufficient. In short the results of both these investigators indicate that in *Dictyota dichotoma* a periodicity in the production and liberation of the sexual products, while seemingly influenced to a marked degree by environmental fluctuations, is primarily due to an inherent periodic tendency on the part of the plant itself.

Whether *Spirogyra* is similar to *Dictyota* in respect to its periodicity has not been definitely settled. Fritsch and Rich ('07) made rather extensive field observations on a number of species of *Spirogyra* and came to the conclusion that the stimulus to conjugation is probably an external one consisting of very complex factors and that it is presumably different for the different species. These observers point out that the same species in the same body of water conjugates at varying times in different seasons and that different species under similar conditions do not behave in like manner during the same season. Brown ('08) likewise made numerous observations on *Spirogyra* and other forms in the field and was led to conclude that these algae grow on indefinitely so long as conditions in the environment do not become adverse. *Spirogyra* conjugates when conditions become "hard enough." Copeland ('09), on the other hand, kept a large number of cultures in aquaria and supplemented a study of them with observations on *Spirogyra* in nature. His results indicate that *Spirogyra* has definite periods of growth and activity and he finds "overwhelming evidence....that

....conjugation results not so much from external as from internal conditions."

Of a somewhat different nature is the work of Benecke ('08) which is entirely experimental in character. This investigator put *Spirogyra communis* in different media (water or salt solutions) in aquaria of various sizes. They were for the most part placed in bright light and kept at a temperature ranging from 12 degrees to 20 degrees C. He found, as his tabulated data show, that in nitrogen-free solutions conjugation took place in a short time. The number of zygotes formed, however, varied somewhat in the different media, distilled water being one of the least favorable. If parallel cultures were run in which $-NH_4$ or $-NO_3$ had been added in appropriate amounts (about .05%) to any of the above media or substituted for one of the constituent salts, no conjugation took place at all but generally a good vegetative growth ensued. As a result of his work Benecke believes that conjugation in *Spirogyra* is due to the failure of ammonium salts which he supposes to be removed from the water by angiosperms which increase in size and abundance as the season advances.

Benecke's work seeming to be of a very definite character, an attempt was made by the writer during the winter of 1909-1910 to repeat these experiments using other species in the place of *S. communis.* The result has been almost a complete lack of conformity in so far as the question of zygospore formation is concerned. The stimulating effect of an ammonium salt on growth, however, frequently proved to be quite as marked as it was with *S. communis* in Benecke's cultures. The species used for the present study were chiefly *S. setiformis, S. longata, S. Grevilleana, S. dubia,* and *S. porticalis.* There was also a large and very resistant form which occurred for the most part as scattered filaments associated with the other species and generally persisting in the aquaria after the associates had died. Unfortunately *S. communis* was not accessible.

In the fall, *S. setiformis* was the most available form. It was found in considerable abundance in one of the small,

shallow, "tropical ponds" at the Garden and remained there till the pond was covered for the winter. Good vegetative material and occasional conjugating filaments could be found throughout the fall but the species did not reappear in the spring, due possibly to interference of the workmen. December 10, some of this material was brought into the laboratory and placed in media made up according to Benecke's formulae for his second series. The solutions are as follows:

No. 1. Distilled water.

No. 2. Tap water (In place of pond water.)

No. 3. Distilled water, 100 cc.

KNO_3,	.05 grm.
$Ca_3P_2O_8$,	.05 "
$Fe_3P_2O_8$,	.05 "
$MgSO_4+7H_2O$,	.05 "

No. 4. Like No. 3, but in place of KNO_3, KCl, .04 grm.

No. 5. Distilled water, 100 cc.

KNO_3,	.05 grm.
$CaCl_2$,	.05 "
$FeSO_4+7H_2O$,	.005 "
$MgSO_4+7H_2O$,	.05 "

It will be observed that Nos. 3 and 5 alone contain NO_3 in solution. When Benecke experimented with *S. communis* in these media he found that in the course of eleven days Nos. 1, 2, and 4 produced zygotes and No. 5 showed abnormal developments while in No. 3 no zygotes were formed but a good vegetative growth took place. In my cultures Nos. 1, 2, and 5 had died by the end of the third week without having conjugated, but most of the filaments in Nos. 3 and 4 were in good condition and had apparently grown slightly. Filaments in these two cultures remained alive in the laboratory under conditions that seemed favorable for conjugation from December 10 till after the first of April when most of them gradually died, no conjugation having taken place in either solution although from Benecke's results it was to have been expected in No. 4.

Solutions No. 3 and No. 4 of Benecke's third series were also used at this time. Their composition is:

No. 3. Tap water, 1500 cc.

NH_4NO_3,	.01%
$CaCl_2$,	.005%
K_2HPO_4,	.005%
$MgSO_4+7H_2O$	.005%
Fe_2Cl_6	1 drop of the standard solution.

No. 4. The same as No. 3 except for the omission of the NH_4NO_3.

The exact formulae given were departed from to the extent of substituting distilled water for tap water, the composition of the latter being unknown. With Benecke No. 3 gave good vegetative growth and No. 4 numerous zygotes, but in this case, although many of the filaments in both cultures lived and apparently grew to a slight extent, no conjugation took place during the five weeks in which the solutions remained unchanged. January 14, some melted snow was added to these cultures. The filaments in solution No. 4 died during February, those in No. 3 persisted until April. There was no conjugation in either. In regard to the several cultures started December 10 it is of interest to note that those in tap and distilled water died, as did also several cultures in which NH_4NO_3 alone was added to the water, while the more elaborate media proved favorable. No. 5 of the second series, here as elsewhere, appeared to be slightly toxic, probably due to the $FeSO_4$ contained. It may be remarked at this point that *S. setiformis* has not seemed to respond to NH_4NO_3 by vegetative growth as have *S. dubia* and *S. longata*. It died in the above mentioned solutions and when a slight trace was added to a culture of five months' standing in No. 4 of the second series, the filaments which previously had been in perfect condition became gnarled and distorted.

One other series in which *S. setiformis* was used may be mentioned. Late in February or early in March zygospores began to germinate in an aquarium where conjugating material had been left in the fall and by the first of April the majority of the filaments were from 15 mm. to 20 mm. in length. All the different salt solutions which Benecke had found to favor or permit conjugation in *S. communis* were made up according to his formulae except that in place of the occasional pond or tap water 100 cc. of twice distilled water that had been further treated with iron hydrate was used in each case. April 5 a few filaments were placed in each of these media and kept in the comparatively uniform temperature and good illumination of the laboratory. April 19, when filaments of the original stock had about doubled in length, only one or two of these cultures showed any growth

at all and in these cases it was very slight. Nevertheless no conjugation had taken place and there was none observed subsequently. Despite the precautions, mycelium developed in these cultures and soon affected the algae.

Spirogyra longata was the most abundant form throughout the greater part of the period during which the work was being carried on and served for a large number of experiments. During January, February, and the early part of March it seemed to be confined to a region near the source of a small stream that arises from an artificial pond in the Garden. Late in March it spread into the pond and down the brook and in April and May became excessively abundant. The environmental conditions certainly varied greatly during this period but only a very little conjugating material could be found, although in the same brook *S. Grevilleana* and possibly also *S. porticalis,* appeared, conjugated, and for the most part disappeared. Despite its apparent hardiness under natural conditions *S. longata* proved rather a difficult species to handle in the laboratory. When brought indoors, especially during the winter, it almost invariably grew rapidly for a few days, then suddenly fragmented and died. It acted in the same manner in a number of media, in bright or subdued light, and under somewhat varied conditions of temperature. Later in the season this tendency was much less pronounced. It was found, however, that even during the winter if flasks containing the cultures were placed at once in the brook the plants did not die and could subsequently be returned to the laboratory where they would then live for a long time. Later it was noticed that the addition of a slight amount of NH_4NO_3 a day or two after the plants were first brought to the laboratory served to prolong their life and stimulate growth, but of course this method could not be used with material intended for experimentation. Attempts to induce conjugation were without success except where the stock material had already shown at least incipient stages before the work was begun. Seventeen of the Benecke solutions were made up in melted snow, *S. longata* was added, and the aquaria (flasks) were kept in the stream from March

29 to April 12 and then in the laboratory for a longer period. Early in the course of this experiment the filaments in solution No. 5 of the second series began to twine around each other and even to entwine themselves, and those of No. 1 of the eleventh series (.005% each of Na_2HPO_4 and NaH_2PO_4) developed very marked false branches which did not resemble conjugation tubes but were much larger and longer. Several of the other cultures showed distortions of a less marked character but in none was there any conjugation whatever. The abnormalities referred to above have appeared from time to time in very different solutions but sufficient data are not at hand to warrant any opinion as to whether they are due to osmotic or toxic factors or represent an incomplete response to some stimulus to conjugation. A number of similar experiments with this form need not be considered beyond noting that Benecke's methods when tried under various conditions uniformly failed to produce conjugation except in the single case to be described in the following paragraph.

April 29, a mass of *S. longata* was found conjugating. Filaments that had already conjugated were pulled out from the rest and the remaining material (A) was used to start four cultures, two in No. 3 of Benecke's second series and two in No. 4. One of each was placed in the laboratory, the others in the brook. Four parallel cultures (B) were identical except that the material was from another mass where no indications of conjugation were to be found. May 10 the material from lot A which was placed in the pond showed vigorous growth in No. 3 and more or less conjugation (but no zygotes) in No. 4. These results approximate those of Benecke, the only clear case so far as this species is concerned. The similar cultures left in the laboratory showed stationary conditions in No. 3 and for the most part death in No. 4. The cultures in lot B showed good growth in the field and an indifferent condition in the laboratory. Although No. 4 in the field at first showed a very few tube-like outpushings these did not develop and no conjugation took place. The eight cultures were then put in the bright light of a south window

and re-examined on May 30 when it was found that all the material from the original lot A was either dead or greatly reduced while the four cultures from lot B which had received identical treatment were in good vegetative condition although there were individual differences between them.

Three other cultures started on April 29 consisted of conjugating material placed in aquaria of 500 cc., each kept in bright light but not in much direct sunshine. No. 1 was pond water which had been strained for the partial removal of animalculae. No. 2 was the same plus .008% each of Na_2HPO_4 and K_2HPO_4. In No. 3 these salts were replaced by NH_4NO_3, .016%. On May 6 the filaments in No. 1 were prostrate but the few zygotes appeared to be normally developed. No. 2 showed excellent vegetative growth in addition to the zygospores. The filaments in No. 3 were growing vegetatively and some of the zygospores appeared normal but many were bright green with a clear space either at one end or in the middle. In these clear spaces rapid Brownian motion such as is characteristic of the vegetative cells could be observed. May 10 apparently no additional zygospores were forming and the aquaria were placed in bright sunlight. By May 30 the filaments in No. 1 were entirely gone, those that were alive in No. 2 were in good condition and apparently taking a new start, and those in No. 3 were at a low ebb, the stimulating effect of the NH_4NO_3 having passed. None of the zygospores had germinated up to this time.

Small amounts of *S. Grevilleana* and *S. porticalis* were available during a part of March and early April. March 18 three sets of solutions were prepared; distilled water and Nos. 3 and 4 of Benecke's second series. These were used as stock solutions and from each of them were started two cultures of *S. longata,* two of *S. Grevilleana* and two of *S. porticalis. S. longata* had not been found fruiting, but both of the other species were conjugating at the time in nature. These cultures were examined on March 22 and it was found that *S. longata* and *S. porticalis* were vegetative throughout with no evidences of conjugation. *S. Grevilleana* likewise was vegetative in distilled water and in both solutions from No. 3, although

in one of these there were slight indications of tubes. But in both cultures of the No. 4 solution there was evident conjugation. Thus one of the three species partially fulfilled the expectations of Benecke's theory, while the other two failed entirely to do so. A larger series gave conjugation in eleven cases out of an expectation of fifteen with *S. Grevilleana,* but no slightest indication of it in parallel cultures of *longata* which were treated in identically the same manner. As was stated above, *S. Grevilleana* was conjugating in nature at the time the observations were made, and consequently it cannot be predicted how it would act during a period when conjugation is not normally occurring. Later in the season, however, when this species had become scarce occasional filaments sometimes occurred in my cultures associated with other forms; at this time, however, they did not conjugate, as would have been expected, but grew vegetatively.

On several occasions masses of actively conjugating *S. dubia* were brought into the laboratory and placed in cultural solutions. After several days a few filaments presenting the normal vegetative appearance could generally be found in all the solutions. The origin of these filaments was not determined with certainty, and while they seemed to be derived from filaments that had in part conjugated, it is not entirely impossible that they were younger plants that would have remained vegetative even in nature. Their subsequent fate in the laboratory varied, but those in the more complex salt solutions generally showed the most vigorous growth, No. 4 of the second series (which Benecke found to favor zygospore formation) being about the most favorable.

The germination of the zygospores in this species is of interest in view of the fact that in some cases it occurred after a very brief time. Zygospores that were forming about April 15 and at that time placed in solution No. 4 of the second series were germinating abundantly by May 3 when some of them had already become three cells long. They were also actively germinating after the same length of time in No. 4 of series ten (.02g. $NaNO_3$ in 150 cc. of water) and to a much less extent in No. 3 of series two. In pond and tap

water, the other two media used in this experiment, young plants could not be found at this time, although by May 30 many of those in the tap water had also germinated. In like manner when conjugating material of the same species, but from a different locality, was placed in solutions Nos. 3 and 4 of Benecke's second series and also in tap water, distilled water, and a solution containing .008% NH_4NO_3 there was, at the end of four weeks, more or less germination of zygospores in each case, although to a less extent in the tap water and distilled water. With the other species a similar germination did not seem to occur. Whether or not *S. dubia* germinated in nature at this time could not be satisfactorily determined, for while no filaments were seen it may have been because they were eaten by the large number of tadpoles that appeared in the ponds.

Briefly to summarize the results obtained, it appears that there are specific differences as regards the reactions of filaments and zygospores in the species studied, and that Benecke's conclusions, based on the reactions of *S. communis,* are probably not of general application, or are applicable only under very special conditions. Of the five species investigated three failed entirely to give the expected results, and a fourth failed in every case but one. The remaining species, *S. Grevilleana,* seems to agree more closely with *S. communis* but even here the agreement is not complete. The existence of sexual strains, such as occur in some of the moulds, seems to be suggested, but evidence on this point is lacking.

When Benecke's results are analyzed it becomes apparent that he did not find any specific stimulus that would induce conjugation unless the absence of ammonium salts be taken as such. The foregoing observation seems to show very clearly that in many cases at least the absence of these salts is not enough to bring about conjugation. Hence it seems all the more probable that, as Fritsch and Rich have stated, the conditions governing conjugation in this genus are very complicated and probably not always of such simple nature as Benecke is inclined to believe. Indeed it is still possible that *Spirogyra* like *Dictyota* is inherently periodic in its func-

tions, although its periodicity may be extensively influenced by the environment.

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THE FUNGOUS ROOT-TUBERCLES OF CEANOTHUS AMERICANUS, ELAEAGNUS ARGENTEA, AND MYRICA CERIFERA.*

BY E. G. ARZBERGER.

INTRODUCTION.

Although considerable work has been done by several investigators on the peculiar root-tubercles found on the alder and some other plants, no satisfactory account of their nature, origin, and function has yet been fully set forth. Especially the question of their relation to the so-called mycorrhiza remains to some extent unsolved. The following studies were undertaken with the hope of getting fuller information regarding the gross structure, physiology and cytology of the forms which occur on the roots of *Ceanothus, Elaeagnus* and *Myrica.*

HISTORICAL.

A brief resumé of the leading views concerning the root tubercles, from a historical standpoint, is interesting from the fact that, for more than three decades, these structures on the alder were described by various investigators, none of whom proceeded far enough to determine the true nature of the tubercles and the fungus which causes them.

Meyen, (23) in 1829, gives the first description of the tubercles on the alder and considers them as "pseudomorphosed roots," in the ends of which there is a parasitic growth comparable to that of *Lathraea, Rafflesia* and *Bálanophora,* though of a more primitive nature and in many respects resembling growths of a parasitic origin, found in an animal body. He claimed that the tubercles are formed when the alders grow near flowing water and in shady places.

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Schacht (32-37) in each of his six productions on plant anatomy and physiology, refers to the tubercles found on the alder. He sets forth their morphology with description and illustrations, and at first states that they are only normal growths, but in later articles he considers them as abnormal growths of the roots; however, no fact or theory regarding an organism which may cause them is presented.

Döbner (8), in his Lehrbuch, mentions the tubercles formed on the roots of alder seedlings and regards them as roots altered by much branching and thickening. No reference is made to parasitism.

Jäger (18) considers the tubercles as insect galls similar to those found on the twigs of the willow and the pine.

Rossmässler (31), in his description of the roots of the alder, mentions the rusty-brown cluster-like outgrowth found on large and small alder plants.

In 1866 Woronin (56) first described and presented figures of the fungus that produces the tubercles on various species of the alder. He considered it closely related to the genus *Schinzia* of Nägeli, and proposed for it the name *Schinzia alni*. Later he made additional investigation on young tubercles and stated that two organisms, one resembling *Plasmodiophora brassicae* and another a filamentous type, may be present in the cells. In the latter view he was supported by Möller (24), who called the fungus *Plasmodiophora alni*.

Ratzeburg (30) refers to the abundance of tubercles on the alders and gives a full description accompanied with plate figures, some of which were borrowed from Woronin. He predicts that an organism belonging to the lower cryptogams will be found in the cells.

Möller (24) supported the views of Woronin regarding the nature of the fungus and proposed the name *Plasmodiophora alni*. Five years later (25, 26) he reinvestigated fresh material and retracted his earlier views in favor of those set forth by Brunchorst (6), who, by a thorough investigation, arrived at the true nature of the fungus in a study of the tubercles of *Alnus* and *Elaeagnus*. With a more modern technique he was able to determine the mycelial nature of

the fungus and its relation to the sporange-like structures which Woronin and Möller considered as spores. Brunchorst found that the content of the sporange segments into several parts which he considers as spores. In the longitudinal section he points out three distinct stages through which the fungus passes in its development. He regarded the fungus as distinct from *Schinza* and *Plasmodiophora* and considered it a new genus, naming it *Frankia subtilis,* though admitting that the fungus varies morphologically in different host plants.

Frank (9, 10), in his first contribution, sets forth some peculiar views in regard to the fungus, considering the tubercles as normal growths for the transitional storage of proteid material. He mentions the vesicles and the various changes which they undergo. In a later publication he revises his former views and in many respects corroborates the conclusions of Brunchorst. He did not succeed in obtaining growths from cultures but describes very clearly the intercellular infection, the effect of the fungus on the cell and its nucleus, and the symbiotic relationship of the host cell and the fungus. However, he did not believe with Brunchorst that the vesicles are fruiting bodies with the dividing contents comparable to spores. As the most appropriate name for the fungus Frank proposed "Ernährungsphysiologische Mycorrhiza."

Atkinson (1) is the first who investigated the root-tubercles of *Ceanothus americanus,* which were discovered in 1890 by Dr. W. J. Beal in Michigan. On examining fresh material he found the organism producing structures closely allied to that which is found in the tubercles of *Alnus, Elaeagnus,* and *Myrica,* hitherto so well known in Europe. He presented in his paper an accurate description of the individual tubercles as regards color, shape, size, method of branching, the formation of large clusters, the various layers of tissue and the cell, finding that the internal structure of the tubercle varies but little in relation to the various tissue systems occurring in the normal root. The vascular cylinder is surrounded by an abnormally developed parenchymatous tissue which contains the parasite, and in *Alnus serrulata,*

cells containing the fungus are only a little if at all larger than the uninfected ones. No radial elongation of cells could be found as in *Ceanothus*. When mature, the fungus forms a dense cluster in the infected cells, the central portion of which is composed of completely branched threads bearing spherical sporangia at their end. Atkinson considers this fungus as a distinct species from all other forms, and proposes the name *Frankia ceanothi*. A symbiotic relationship between the host and parasite seems probable to Atkinson from the fact that the plant appears to suffer in no respect from the infection.

It was left for Hiltner (16) to prove experimentally that the root tubercles on the alder enable the plant to assimilate the free nitrogen of the air by a process analogous to that which occurs in leguminous plants. Furthermore he showed that alder plants can grow without tubercles provided the solution or soil in which they grow contains nitrogen in some form, and conversely that the growth of the tubercles is inhibited when the plants are grown in a solution or soil where nitrogen is present in abundance. Calcium nitrate entirely stopped the growth of the tubercles.

Subsequent to the above investigation, the attention of Nobbe and Hiltner (28) was called to *Podocarpus,* an oriental conifer, which possesses a large number of root-tubercles caused by an endotrophic mycorrhiza. Guided by their experiment on the alder they carried on investigations with greenhouse plants grown in quartz sand to which only non-nitrogenous culture solutions were added. For five years before their results were published the plants grew luxuriantly and they concluded that they obtained the required nitrogen from the air.

Although Schacht (32), Brunchorst, Möller and a few other earlier investigators had examined the root-tubercle-like structures which are found on the roots of several *Cycas* species grown in green-houses, it was not until 1901 that Life (20) gave a more detailed description of the tubercles, noting that a fungus, a bacterium, and an alga inhabit these structures. In young tubercles and in the tips of older ones, he found only the fungus and bacteria, which, he claims, pre-

pare the way for the blue green alga. Lenticels also occur on the tubercles, which may serve in aerating the root system. Life believed that the tubercles may aid in free nitrogen assimilation for the plant.

Because of the evidence that the fungus living in the tubercles of *Podocarpus, Alnus* and *Elaeagnus,* aids in the fixation of free nitrogen for the use of these plants, Hiltner (17) was led to investigate *Lolium temulentum,* with which, according to Vogel and Nestler, there is associated a fungus that inhabits all the tissues of the plant even including the seed. *Lolium italicum,* on the other hand, contains no fungus. Plants of both species were grown to maturity in nitrogen-containing and nitrogen-free soil with respective controls. Chemical analysis showed that *Lolium temulentum* grown in nitrogen-free sand contained far more nitrogen than was originally present in the seed, and the plants nourished with nitrogen compounds contained nitrogen far in excess of that which was supplied by the solution; hence Hiltner claimed that the free nitrogen of the air is used by this fungus-containing plant. Luxuriant growth of other plants likewise attacked by fungi was considered as evidence in favor of this view, but it has been shown by Brefeld (4) that such is not the case with the Ustilagineae and it remains to be proven for other fungi.

Probably one of the most thorough investigations made on an endotrophic mycorrhiza in relation to its host cell is that of Magnus (22), who sets forth very fully the facts found in the rhizome and root of *Neottia Nidus avis,* where a fungus enters from without and fills a definite concentric layer of cells. All the infected cells do not react alike, for in certain ones the fungus destroys the protoplasm and forms organs for the purpose of maintaining itself during the winter and for the infection of new cells in spring, while in other cells the fungus, after having attained a certain development, is digested for the nourishment of the host, and the undigestable portion remains and collects in a mass in the center or near the cell wall where layers of cellulose are formed around it. The nuclei of host and digestive cells furnish various phenomena which are elaborately described.

Shibata (38) made a cytological study of the endotrophic mycorrhiza found in the tubercles of *Podocarpus chinensis* and *Alnus incana* and in the rhizome of *Psilotum triquetrum*. In the *Podocarpus* tubercles he found a large hyphomycete, which, by branching, filled the entire cell. The host nucleus increases in volume and assumes an amoeboid form, whereupon it divides mitotically, frequently forming as many as eight smaller nuclei which become distributed in the cell and again become amoeboid in form. When the fungus has attained its full growth it is digested by the host cell and the nuclei may resume normal conditions and divide mitotically. No cell walls are formed subsequent to nuclear division, the cells ultimately degenerating with the disintegrating tubercle. Regarding the symbiotic relationship, Shibata corroborates the views held by Magnus (22) and Frank (10), who have shown that the fungus is subservient to the host cell. Shibata showed further by experiment that a proteolytic enzyme capable of digesting fibrin is present in the tubercle.

In the rhizome of *Psilotum* he found the conditions quite similar to those in *Podocarpus,* except that the fungus mycelium confines itself to the periphery of the cell, and the host nucleus undergoes no division. The undigested portions of the fungus form a dense aggregation in the center of the cell where it is cemented together by an amyloid-like substance and finally surrounded by a membrane. This is quite similar to the conditions in *Neottia Nidus avis.* By means of descriptions and figures, Shibata presents the true conditions as they are found in the tubercle of the alder. Here in addition to what earlier investigators had found, he mentions a dense clump of cytoplasm which remains after the absorption of the fungus in which small spherical and heavy stained bodies appear to which he applies the term "Sekretkörperchen," similar to those bodies which Heidenhain found in the gland cells of several animals. These he claims are instrumental in the production of an enzyme in the cell which dissolves the fungus.

Björkenheim (2) in one of the most recent papers on the alder tubercles presents the old facts in a somewhat different

light without, however, contributing anything new. He finds very large hyphae in the young tubercles, some measuring 4–5 μ in width, which become finer as the tubercles grow older. His other facts regarding the fungus and the host cell are similar to those described by earlier investigators.

Wolpert (54) made a study of the tubercles on *Alnus alnobetulina* and corroborates the results of Brunchorst and Shibata, in addition showing intercalary and apical swelling of the septate hyphae, conditions which are similar to the enlarged hyphae found in *Psilotum*. A view not earlier advanced is that the angular structures in the sporanges germinate and form new hyphae, but from the text figures given it would appear to be an artifact rather than an actual germination. The so-called "Secretkörperchen" described by Shibata were not found in those cells where the fungus is digested.

THE TUBERCLES OF CEANOTHUS.

For cytological study the root-tubercles of *Ceanothus americanus* were gathered from plants growing in the open woods surrounding Madison, Wisconsin, the material for subsequent investigations being collected from plants growing in Forest Park, and in other open woods on the outskirts of St. Louis, Missouri. Because the tubercles dry very easily on exposure, fixations were made in the field in order to obtain as nearly perfect material as possible. A few plants, however, were removed from the soil and transplanted into flower pots which were kept in the greenhouses, where the roots were kept in good condition for a reasonable time, packed in a large amount of moist sphagnum.

Judging from the material gathered at various sections of a state as well as from different states the indications are that the species of *Ceanothus* are everywhere affected with tubercle growths. *Ceanothus americanus* and *ovatus*, common in Wisconsin, as well as *azureus, Delilianus, Fendleri* and *microphyllus*, species native to the southwestern United States, which are now growing in the Missouri Botanical Garden, were all examined and found to possess tubercles.

Thus the formation of these tubercles is not confined to any definite locality nor to any special species of the genus.

Of the various fixing solutions used, Kaiser's sublimate-acetic and iridium chloride gave the best results. After fixation the material was hardened and imbedded in the usual manner and transverse and longitudinal sections 5–8 in thickness were made. For staining, Fleming's triple and Heidenhain's iron haematoxylin stains were used, the triple stain proving the better for differentiating the various host tissues, the parts of the cell and the mycelium of the fungus. The nuclear structure of the fungus could best be determined with the iron haematoxylin stain, although the grosser structure, such as the cell wall of the mycelium, is not as distinct. Serial sections prepared with these stains afforded the best means for the study of the minute structure of the fungus and the host, although examinations of fresh material were made in an attempt to obtain pure cultures, and the testing of the enzymes present in the tubercles of course was done with living material. The result of this work is deferred to a subsequent paragraph.

External Characters.

All the plants of *Ceanothus americanus* examined possessed tubercles on their roots; the youngest plants have only a few, often not more than two or three, whereas older plants have a large number; one nine years old possessing 1,830 tubercles on the roots. The number thus increases with the age of the plant and with the root mass, a fact contrary to conditions found in the alder, where very old trees do not seem to have as many as the younger and more rapid growing ones. The tubercles are also found at a greater depth than with the alder, the greatest number and the largest coral-like clusters being usually found 4-10 inches below the surface of the soil. When there are but a few lateral roots the tubercles are frequently found arranged along the tap root, often to a depth of 1.5 to 2 feet, penetrating the hard subsoil, but only individual tubercles or small clusters are found at such depths; the larger masses are found nearer the surface in the looser soil.

It may be noted that the greatest number of tubercles either branched or unbranched are attached by a small root to a larger one, it seldom happening that the tubercles or clusters are attached close to a large root. Often a cluster is formed by a number of rootlets, which are closely packed together, each of which may have several tubercles, so that the irregular masses composed of unbranched individual tubercles are probably caused by the infection of many adjacent rootlets.

The youngest tubercles all originate from the sides of fine thread-like roots (pl. 6), where their beginning may be noted as small protuberances on the sides, infection having probably taken place through an epidermal cell or a root hair. Instances are very rare where a tubercle, or cluster of them, terminates a root so as to result in such massive structures as on the alder. At first the young tubercle is merely a bulging of the epidermal tissue, subsequently the vascular cylinder sends off a branch toward the infected region and the young tubercle becomes spherical, then ovoid and finally elongates into a cylinder as the vascular tissue increases in growth. The average diameter of an ordinary tubercle is from 1 to 1.5 mm. and in one year's growth it may attain from 3–6 mm. in length. The growth in length continues from year to year so that some may become 11–14 mm. long, but they still retain their original diameter. Through di- and tri-chotomous branching the loose cluster is formed on a single rootlet, and one of these may ultimately result in a mass 4–6 cm. in diameter, all of which originated from a single tubercle (pl. 6). There is some variation in the color of the tubercle, due to the nature of the soil in which these plants grow. As a rule the youngest tubercles are light gray, some are almost perfectly white, becoming pinkish as they grow older, while the older ones are flesh colored, becoming darker with age. Atkinson (1) describes the very youngest as having a flesh color, probably due to his not having found the very earliest stages of the tubercles. There is no pigment in the outer layer of cells, such as is abundantly present in the alder tubercles.

Internal Structure.

A study of the internal structure of the tubercle was made from both longitudinal and transverse sections (f. 1), the smallest tubercle being found to possess the various tissues in common with older ones, only in a more rudimentary form. The tubercle consists of the outer corky layer, the outer and inner cortex and the vascular cylinder. Of these tissues the cortical parenchyma makes up the greater bulk of both small and large tubercles, while the vascular cylinder is quite narrow, and does not extend far into the young tubercle (f. 1). The corky layer is made up of several thick walled cells the outer of which are usually broken. Beneath this is a layer of tissue made up of from four to five layers of oblong thick-walled cells, between which and the vascular cylinder is the parenchyma, composed, under normal conditions, of thin walled rounded cells. The vascular cylinder, bounded by an endodermis, consists of a few xylem strands, a large number of phloem cells and some supporting tissue which surrounds both (f. 1). Many resemblances may be noted to the structures of alder, *Elaeagnus* and *Myrica.*

The fungus confines itself to a zone of tissue two or three layers from the cylinder and from eight to ten layers from the outside of the tubercle, an arrangement strictly adhered to in these tubercles, so that no such irregular structure is produced as in the alder. Thus the fungus makes its home in a cylindrical zone extending from the growing point to the base of the tubercle; in older tubercles the infected belt widens considerably, often containing ten to twelve layers of cells in a transverse section, while the vascular cylinder also grows longer and wider and sends a branch into each division of the tubercle.

The developmental stages of the fungus and the effect on the host cell are best studied from a longitudinal section where the various stages, from the apex to the base, are easily recognized, and inasmuch as general cell infection occurs at the apex of the tubercle, the most abundant youngest stages are found in this region. Although internal infection is most prominent in this region it is also found to occur

in other portions of the tubercle as well. From the infected cells containing a mass of mycelial threads a stout hypha passes out, dissolves its way through the cell wall, and passes into the adjacent cell, directing itself toward the cell nucleus, often pushing it aside from its normal position or else twining about it (f. 2). The infecting hypha is usually quite stout, more so than those of the much branched masses (f. 3, 4), a provision evidently necessary because a delicate hypha would not be able to make its way through the wall readily, and even if it did, there would probably not be enough protoplasm present to initiate a good growth in the new host cell.

At this early stage the mycelium is densely filled with protoplasm in which are imbedded many small nuclei showing very prominently with the haematoxylin stain although the walls of the hyphae cannot be seen (f. 5, 6). After having established itself, short branches originate from the first hypha which in turn branch very abundantly and fill the greater part of the host cell (f. 4).

When the fungus enters the cell there is a stimulation to growth of the cell wall and the protoplast; adjacent uninfected cells are comparatively small (f. 8). The cytoplasm increases and becomes densely aggregated about the nucleus or about the fungal material, staining a very light orange, producing a contrast between the cytoplasm of infected and uninfected cells. All traces of starch grains which are abundant in uninfected cells, have entirely disappeared and even normal adjacent cells suffer the loss of their starch and cytoplasmic content from the infection of their neighbors (f. 2). The cell walls of the host are dissolved at quite an early stage and often four or more cells break down, thus increasing the space which the fungus ultimately fills. The original content of such cells is absorbed very quickly. No multinucleated cells are formed in this manner, but the nucleus of the cell in which infection originated grows very rapidly with the fungus and remains the prominent part of the host cell. The nuclei of the adjoining cells, which are thus brought in contact with the infected cell, disintegrate very readily, probably being used as food by the fungus.

Shibata has found multinucleated cells in the tubercles of *Podocarpus* produced by the original nucleus dividing amitotically when thus stimulated by an infecting fungus, but no such condition is apparent in *Ceanothus*. It should also be noted that some cells not directly connected with infected ones have but little protoplasmic content. Either the fungus robs the adjacent cells in some unknown way, or else the infected cells obtain the lion's share of food supplied by the plant and finally starve the other and smaller cells.

As the host cell increases in size the nucleocytoplasmic relationship is maintained and therefore the nucleus increases with the cell, becoming enormously large compared with the nucleus of an uninfected cell. The average diameter of a normal nucleus is about 5.6μ, whereas the hypertrophied nuclei are usually oblong, measuring $14 \times 21\mu$. Some peculiar and amoeba-like nuclei are shown in f. 8, many of which are quite similar to those in *Podocarpus* which Shibata (37) found dividing directly. Even the nucleolus increases its size in relation to the nucleus and in some instances seems even to exceed its natural proportion. The amount of chromatin also increases and it stains a deep color with gentian violet. Passing farther toward the base of the tubercle, the cell and its contents become hypertrophied to the highest degree. In cross section the large infected cells are arranged radially (f. 1), showing in a longitudinal view a more isodiametric form. Those of the transverse section measure $60 \times 113\mu$, whereas those of a longitudinal tangential section are about 50–60μ in diameter. Concurrently with the growth of the cell the mycelium becomes more and more entwined, branching and frequently broken in many places. The mycelial threads now become much finer and the small nuclei are distributed throughout the length (f. 4, 5, 6). This mass of mycelium, however, never becomes so large that it fills the entire cell since a provision must be made for the next following stage in which the pear-shaped vesicles are formed on the ends of the hyphal branches (f. 7). This is confined to a definite region, best seen in the longitudinal section where it stains deeply with gentian violet and very dark with haematoxylin, a fact indicating that there is pres-

ent an abundance of chromatin material in the fungus at this stage. Generally this zone appears in the middle of the young tubercle. The pear-shaped or spherical vesicles, packed closely together around the periphery of the cell, are the swollen ends of hyphae densely filled with protoplasm. In preparing the sections they are often torn loose from the large mass, though still retaining a portion of the hypha (f. 10, 11). They are quite similar both in shape and content to the vesicles found in the tubercle of the alder, except that in the *Ceanothus* form there is no double wall such as is pointed out in the alder by several investigators. At first the vesicles are filled with a dense protoplasm containing one nucleus-like body (f. 10), which at a later stage increase in size. Subsequently the material in the vesicle divides into two parts with a very faintly stained substance between them. Apparently this is the mature condition of the fungus and its ultimate products are contained in the sporange-like structure. Although not surrounded by a distinct and perceptible wall these parts are quite analogous to spores but the final fate of these structures and their relation to the infection of the plant could not be determined.

At this stage every trace of starch and cytoplasm in the host cell has disappeared, its nucleus becoming very irregular and shriveled, staining a light orange and usually lying near the periphery of the fungal mass (f. 7, 9). However, the nucleole is still quite large, its appearance indicating that it retains its vitality. Thus all the protoplasm of the cell is used by the fungus to build up its structure, and not until there is no more protoplasm in the cell does the degeneration of the fungus begin. This is shown by the gradual contracting of the entire fungal mass and the collapse of the walls of the mycelium and vesicles, although occasionally one or two vesicles may be found which are unaffected (f. 13). All signs of nuclei in the fungus disappear and the only thing remaining is the rigid cell wall. At a certain stage in this struggle between the fungus and its host, one would infer that the fungus is the victor, and it may be temporarily, yet its life, after having attained this stage of development, is relatively short and its death is brought about by starvation.

This is quite different from the conditions in the alder (38) and *Neottia Nidus avis* (22), where the fungus is finally absorbed by the so-called digestive cell and the nucleus resumes its natural processes. However, it seems that the normal adjoining cells may utilize, in some manner, the remaining protoplasmic material of the fungus. The turgidity of the host cell becomes less and less and the surrounding cells crowd it into a smaller space, so that none of the earlier structures of the fungus or of the host can be recognized. No nucleus ever reappears, and thus the history of host cell and fungus ends in the death of both. Though Shibata has described, in *Alnus,* certain protoplasmic structures, called "Secretkörperchen," which in some way dissolve the fungus so that it may be utilized as food by the host cell, and Zach (58) has noted similar conditions in *Elaeagnus* as well as in the alder tubercles, nothing of such a nature occurs in *Ceanothus.*

THE ROOT TUBERCLES OF ELAEAGNUS.

Warming (52) was the first to give a full account of the tubercles occurring on the various genera of the *Elaeagnus* family, although he credits Jörgensen with being the first to note the tubercles on the roots of *Elaeagnus, Hippophäe* and *Shepherdia.* Accompanied with a text figure he presents a morphological description of the tubercles. Their cause, he attributes to a parasitic myxomycete resembling *Plasmodiophora brassicae.* The numerous spherical bodies are mentioned and he claims that they resemble spores or are identical with them.

Later Brunchorst (6) made an intensive study of the tubercles of *Elaeagnus angustifolia* and *Hippophäe rhamnoides* in connection with those of *Alnus glutinosa.* He considers the fungus to be the same in both plants and that whatever facts he presents regarding the alder are also true of *Elaeagnus.*

More recently Zach (58) has also made a comparative study of the tubercles on *Elaeagnus angustifolia* and *Alnus glutinosa* in which he points out that the hyphomycete in both plants belongs to the same genus and species: viz: *Frankia*

subtilis Brunchorst. He makes no distinction between the two host cells as they are affected by the fungus, but states that the broken threads, "Stäbchen," so-called by Shibata (38), are the concentrated cell content of the hyphae, the cell wall being unstained. Zach finds spore-like knots and bacteria-like threads which are degenerate forms of hyphae. These he claims absorb a great deal of water and thus fill the entire cell lumen. The terminal swellings of the hyphae are also degenerate stages of the fungus which are ultimately digested by the host cell, during which process the fungal masses pass through various degenerative stages. Spherical, oval and other shaped bodies, of an oily consistency, appear during the digestive process and to these he applies the name "Exkretkörper," though no such bodies as described by Shibata were found.

Zach (60) finds similar conditions in the host cell of the tubercles of *Cycas revoluta,* in which he recognizes a phenomenon comparable to phagocytosis amongst animal cells.

Because the root-tubercles which occur on *Elaeagnus* have been reported by several investigators as resembling in many respects those of the alder, the present investigation was undertaken with *Elaeagnus argentea,* and comparisons were made with the more recent research carried on with alder tubercles as well as those of *Ceanothus* and *Myrica.* The tubercles were gathered in the autumn of 1909 from plants growing in the Missouri Botanical Garden. It was found that they do not occur as abundantly as on the alder and *Ceanothus* and, furthermore, are located on roots much deeper below the surface than in the alder or *Ceanothus.* This, however, may be due to the fact that the *Elaeagnus* has its roots set much deeper in the soil. All plants of this species are more or less infected, yet some have far more than others. *Hippophäe rhamnoides* and *Shepherdia canadensis,* two other representatives of this family, were examined and both found to possess tubercles quite similar to those of *Elaeagnus argentea.* Thus all the genera of this family grown in the garden have tubercles on their roots.

No such large masses or clusters of tubercles as occur on the alder are found, although they vary in size from a few in-

dividual tubercles to much branched structures, the largest of which was 3–5 cm. in diameter. The larger clusters are very compact and usually attached very close to a large root (pl. 7). An unbranched individual tubercle may be described as a cylindrical structure, 8 to 1 mm. in diameter and about 5 to 6 mm. in length, terminating in a smooth rounded tip having no indication of a root cap. Very loose clusters, however, may have tubercles which are much longer. After a tubercle has attained a certain length, branching takes place in the tip which may continue until ultimately 24 to 30 branches are formed, the origin of which may be traced back to a single individual.

The color of the youngest tubercles is a dark gray, becoming much darker near the base. Old tubercles are dark brown or almost black, with a grayish colored tip. This dark color is due, to some extent, to the decaying cortical layer which peels off, thus giving the rough appearance. Individual tubercles of all sizes were fixed in Kaiser's sublimate acetic and Fleming's weaker and medium solutions. Specimens fixed in corrosive sublimate solutions gave the best results; the osmic acid solution so hardened the material that sectioning is difficult. From the imbedded material longitudinal and transverse sections, 5–10μ in thickness, were made, which were stained with Fleming's triple and Heidenhain's iron haematoxylin, but the triple stain set forth clearly all cytoplasmic and nuclear structures of both the host and fungal cells.

The morphological structure of the tubercle is best studied from a median longitudinal section in which the various tissues are shown as well as the different stages of development which the fungus passes through in its limited life cycle. All the tubercles retain to some extent the typical tissue systems of a normal root, the cortical parenchyma being greatly enlarged from the hypertrophy which is primarily brought about by the fungus which lives in the cells. The chief tissues of the tubercle are the inner and outer cortex and the vascular cylinder, which is bounded by a thick walled endodermis (f. 14). The outer cortex consists of oblong cells, the walls of which stain a deeper color with gentian violet, while the

cells of the inner cortex have quite thin walls and are more isodiametric excepting the infected ones which are usually elongated radially. The infected region, as shown in f. 14, is located in the middle portion of the cortex, and the distribution is more uniform than in the alder, being quite similar to conditions in *Ceanothus*. Usually four to five layers of cells outside of the central cylinder remain uninfected, although there is some tendency for the fungus to penetrate inwardly. Many cells are found which are just being infected quite near to the vascular cylinder, but in a young tubercle a comparatively thick layer of uninfected tissue remains outside of the vascular cylinder. In a large mature tubercle the vascular cylinder composes about one-fifth of the diameter and extends quite far into the tip. It is quite uniform in width, except that it tapers somewhat toward the end. Beside the endodermis which surrounds it, the cylinder consists of a few strands of xylem, still somewhat radially arranged, and with phloem cells between them. The bundles are bounded by parenchymatous cells, a number of which fill up the central region of the cylinder.

A study of the developmental stages of the fungus may best be made from a longitudinal section in which the various stages can be determined. Beginning with the apex and continuing toward the base of the tubercle, serial stages may be selected which represent the entire history of the fungus in the various cells, the youngest stages being found in the growing region, whereas the oldest may be noted in cells at the base of the tubercle.

An uninfected host cell is usually isodiametric and contains many large starch grains imbedded in a thin granular cytoplasm. Occasionally a heavy staining substance is met with which, however, has more of an intercellular than intracellular appearance, and is much like the tannin met with in the alder tubercles. The cell nucleus is relatively large and filled with chromatin in a fine reticulated stage (f. 16, 17, 19).

Infection takes place acropetally; the fungus passes from cell to cell by forcing its way through the cell wall (f. 16) and frequently several hyphae may be noted, placed along the inside of the wall for a considerable distance before one

or all pass out of the cell (f. 18). Apparently the fungus secretes some enzyme which dissolves the cellulose wall and thus prepares the way for the infecting hyphae. Upon entering, the fungus directs its hyphae toward the nucleus, then it builds up a dense tangled mass on one side, frequently crowding the nucleus out of its original position. In many of the infected cells the nucleus lies close to the cell wall and the fungal mycelium nearly fills the remaining space. Figure 19 shows where such a condition is just beginning. Again, other stages are found where the nucleus has retained the central position in the cell and the fungus builds its structure entirely about it (f. 16, 19).

When the fungus enters a cell the first noticeable feature is the increase in size of the nucleus, then follow the growth of the cell wall, the disappearance of the grains of starch and the formation of dense cytoplasmic masses in various parts of the cell. The latter may be comparable to that substance in the host cell which Zach (58) calls the "Exkretkörperchen." No indications are found in any of the *Elaeagnus* material examined that large portions of intercellular walls are dissolved in order that a larger space may be provided for the fungus; a phenomenon invariably found in the tubercles of *Ceanothus.* In *Elaeagnus* the cell walls may be more resistant, or when the fungus is once inside the cell it seems as if there are no more secretions which will break down the walls of the host cell. The normal cells adjacent to the infected ones show no signs of being affected in any way by their neighbors at least as far as cell content is concerned.

With the increase in size of the cell and its nucleus, the nucleo-cytoplasmic relationship is constantly maintained. Exact measurements could not be made because of the irregular form of nucleus and the cell; yet measurements as nearly accurate as possible indicate that a definite ratio exists between the size of the nucleus and that of the cell. All nuclei of the infected cells, which at first are spherical often containing several nuclei, are in the so-called resting stage, the chromatin being distributed in very fine masses; frequently this is very dense around the periphery of the nuclear membrane. The

size of the hypertrophied cells varies from 56 to 91×70 to 112μ and the nuclei found in them measure 15 to 20×27μ, while some of the larger spherical ones are only 16 to 20μ, in diameter, though with a nucleolus measuring 5 to 9μ.

At this stage the fungus has attained its greatest vegetative growth and almost fills the entire host cell; a condition which may be comparable to that found by Magnus (22) in *Neottia*. It is likewise at this stage that the host nucleus acquires its greatest volume; the cytoplasm filling every possible space in the cell which is not occupied by the fungus (f. 19). The symbiotic relationship is now very evident, both the host and its guest seeming to prosper for a definite period.

Following the mycelial stage, the ends of the fungal branches begin to swell, forming spherical or pear-shaped bodies which much resemble the fruiting sporanges of a higher fungus. These "vesicles," as they have been called by some investigators, are relatively small, the largest being 2.8 to 3μ wide, whereas the mycelium on which they are borne is only .2 to .3μ in width, and in fresh material the mycelial threads are so delicate that the sporange-like structures break off very readily (f. 19, 20, 21). The young vesicles are filled with granular cytoplasm in which irregular and dark staining bodies may be found (f. 20, 21), and later the content segments into halves, quarters and even smaller portions, in many of which a small nucleus may be determined. This process is quite analogous to spore formation among higher types of fungi, yet no stage can be found where there is a definite rounding up of the spore-like mass into structures with definite walls. The formation of walls may be inhibited because of the parasitic nature of the fungus, or the fungus may be of that primitive type where no thick wall is formed around the spore.

It is at this stage that the host nucleus assumes an amoeboid shape, frequently becoming very irregular, and portions project quite a distance into the fungal mass (f. 19, 25), a condition probably due to lack of space in which to round out more uniformly. This condition gives some indication of the host cell assuming a digestive function although not

so effective as the digestive cells of *Alnus,* orchids, and *Podocarpus.*

After the development of the sporanges the fungus, as an entire mass, begins to collapse, the sporanges break open and their walls, which stain dark with the triple and haematoxylin, appear as mere shell-like coverings. All of the indications are in favor of the view that the content of these sporanges has escaped but whether in the form of a swarm spore or otherwise could not be determined. When the fungus is in this stage the host cells contain very irregular nuclei (f. 26), and serial stages may be noted where the nuclear content gradually dwindles away until finally nothing but the nucleolus and the nuclear membrane remain; and these too ultimately disappear. Thus there is a long continued struggle between the host and the fungus resulting finally in the destruction of the cell, the nucleus being its most resistant part. The question may arise whether the cell might not still be living after the nucleus is destroyed; all indications are negative, however, being quite similar to those which Gerassimoff (11) obtained with enucleated *Spirogyra* cells which had but a short life even though the cytoplasm was left in almost a normal condition. Just how far the fungus and the host have been mutually injurious, and which one outlives the other, is a difficult question to answer. If the destruction of the nucleus signifies the ceasing of cell activity, then the host cell is destroyed by the fungus which in turn dies from starvation. According to Zach (60) the host cell destroys or digests the fungus, leaving but a portion of undigestible material to which he applies the term "Exkretkörper," but no statement is made as to what the cell does after it has gone through this digestive process. Similar conditions are found in the tubercles of *Cycas,* but here Zach points out that both host cell and fungus may be destroyed. Certainly in *Elaeagnus* the host cell and the fungus both die as a result of their relationship and there is no indication of such a perfect symbiosis as occurs in the alder and *Podocarpus,* although it may be true that the normal cells surrounding the infected ones derive some slight benefit from the fungus. Long after the apparent de-

struction of the host cell the fungus gradually loses its content until finally nothing remains but the walls of the hyphae.

Granting the fact that the host cell is destroyed before the fungus, the gradual disappearance of the latter must be caused by agencies outside of that particular cell, for if it were but a ceasing of living conditions the various stainable parts of the fungus would remain in the cell for a long time. However, numerous examinations of cells of this kind from the oldest portions of the tubercle show only walls closely packed together by the collapsing of the host cells where they undergo no further change (f. 23). Whatever benefit the host plant derives from the fungus must be obtained through the host cell while it is yet in a living condition, or by the other living cells which adjoin the infected ones. Possibly the plant can acquire greater gain by suffering the loss of a few cells for the good of many.

THE TUBERCLES OF MYRICA.

The amount of research that has been done on the tubercles of *Myrica* is rather limited when it is compared with all that may be found on the alder; due probably to the fact that they were considered, for some time, to be caused by a similar fungus. Brunchorst (6) was the first to mention the tubercles on *Myrica Gale* and considered the fungus producing them so much like that in the alder that he called it *Frankia subtilis.* Later, Möller (26) found that it differed considerably and made it a new species which he called *Frankia Brunchorstii.*

Shibata (38) has made the most thorough investigation on the root-tubercles of *Myrica rubra,* his observations being on fresh material and on some prepared according to modern cytological technique. He describes the external and internal morphology of the tubercle, noting that the fungus confines itself to a definite region, thus differing from the condition found in the alder and other forms. The differentiation of the tissues begins in the meristematic region, where internal infection of the young cells takes place. At first the fungus consists of a few mycelial threads which give rise to

branches arranged in an aster-like form and ultimately filling the entire cell. Shibata places this fungus with the genus *Actinomyces,* thus differing from that found in the *Podocarpus, Psilotum, Alnus* and *Elaeagnus* tubercles.

Harshberger (15) was the first to report the tuberculous outgrowths on adventitious roots of *Myrica cerifera,* although Brunchorst and Shibata had already made some investigations on *Myrica Gale* and *M. rubra.* He calls them mycodomatia, a term used by Tubeuf (52) for these structures. The tubercles are found on these roots when the stems are surrounded by shifting sand. At first the masses are relatively simple, but by continual branching and growth, aggregations attaining the size of a walnut are produced. The structure of the tubercle was studied from dry material which had been boiled with water and then treated with alcohol. From sections obtained from these he describes the various parts of the tubercle, finding a unicellular hyphomycete, which confines itself to a definite region, infecting cells anew by passing through their walls and forming a dense mass of hyphae within. However, the microphotographs obtained from these sections do not show the true nature of the fungus and Harshberger, rather unwarrantedly, claims for the fungus a position closely related to the *Oomycetes.*

The root-tubercle-like structures on *Myrica cerifera* were gathered in November and December on plants growing in the Missouri Botanical Garden. *Myrica Gale* and *M. asplenifolia* were also examined, but these did not afford as large or as abundant material as the above named species. These structures occur on adventitious roots which grow out from the lower part of the stem, or from branches or stems which have been covered over with leaf mould or soil for several years. This fact agrees with the observations of Harshberger (14), who found these growths on stems which were surrounded by sand in localities where sand dunes are formed. Other roots have a few tubercles which, however, do not attain so large size as the ones that occur on the adventitious roots, nor are they as abundant as the tubercles found on *Alnus, Ceanothus* and *Elaeagnus.*

The tubercles are usually found in masses varying in size

from a pea to that of a walnut (pl. 8). Specimens of the large size were always associated with large stems. Those found on stems three years old had attained a mass 1 to 1.5 cm. in diameter. The cluster is usually very compact and resembles somewhat that of the alder and *Elaeagnus,* although differing in this respect from *Ceanothus.* Harshberger claims that the tubercles are of very slow growth and tries to determine the age of a cluster by comparing it with the age of the plant on which it is found, but his estimate can be only relative. The masses shown in the photograph (pl. 8), growing on plants three years old, have attained 2 cm. in diameter. According to his statement some plants were twenty years old. Granting that growth be uniform, and if three years' growth will produce a mass 2 cm. in diameter, on a plant twenty years old, one would expect a cluster a half a foot in diameter, a size not attained on any plant observed by either Harshberger or myself.

The individual tubercle may be described as a short thickened rootlike structure which branches di- or trichotomously after having attained a certain length. The longest individual branches found were 2 mm. in length. Their thickness varies from .5 to 1 mm., the older portions, however, being the thickest. One peculiarity of these tubercles is that, after having attained a certain length, their tips grow out into a narrow thread-like structure, often attaining 1.5 to 3 cm. in length (f. 28). This again sends out lateral branches which may be found entwined amongst the roots and grass blades. In vigorous growing material the outgrowths of this kind appear quite like ordinary roots of the plant except that their shape is more tapering toward the tip. The structure of the tubercle will be described in detail in a subsequent paragraph.

The color of the youngest tubercles is a light gray to pink changing to a flesh color with age. The long slender tips, nearly colorless when young, become brown as they dry, through exposure to the air and soil. The very old and dry tubercles are dark brown or even black, a color which was attributed to them by Harshberger, who made his investigation with dried material only.

The tubercles originate from the small adventitious roots which do not attain more than several centimeters in length when infection takes place. Many roothairs occur on these roots, and it is quite probable that the fungus, in some form, makes its inroad through them or by some epidermal cell of the growing root. The formation of a mass of tubercles ends the growth of that particular root, where probably the food material all passes into the tubercles.

The material for investigation was taken from living plants, and after a thorough washing was fixed in various solutions, of which Kaiser's sublimate-acetic and Fleming's weaker fluid proved to be the better fixative for these structures. After fixation, the ordinary procedure of dehydrating, hardening and imbedding was followed, though a much longer time was given for infiltration than is usually given for tissues of a softer texture. Tangential, median and transverse sections 5 to 6μ in thickness were made and stained with Fleming's triple and Heidenhain's iron haematoxylin. The haematoxylin stains the nuclear structures of the fungus, but the mycelial wall and different parts of the host cell can be made out with difficulty. Most of the drawings were made from sections prepared only with the triple stain.

The various tissues which compose the tubercle may best be studied from a transverse section. The tubercle is covered on the outside by an epidermis which is usually broken and very irregular except in very young forms. Underneath lies a thick layer of cortex, made up of rather thin walled parenchymatous cells. The outer portion of this contains narrow oblong cells which make up a layer from 4 to 5 cells in thickness. Inside of this there is a layer of cells almost isodiametric, with very thin walls. Farther toward the interior, the cells become larger and radially elongated, some measuring 20 to 25 $\times$ 28 to 45μ. These cells contain the parasitic fungus, forming a definite region two or three cell layers in thickness. Adjoining it on the inside is a region of smaller cells mostly isodiametric with a few oblong ones scattered irregularly amongst them.

The vascular cylinder is bounded by an endodermis composed of small oval thick-walled cells which stain quite

deeply with the gentian violet. The structure of the vascular cylinder does not differ materially from that of the normal root. In the young tubercles the xylem and phloem are arranged alternately and radially, with a few pith cells in the center. The phloem, however, makes up the greater bulk of the tissue.

In the young tubercles the vascular cylinder does not extend far into the apex, whereas in older ones the cylinder with some cortical cells surrounding it, grows out into a slender thread from which lateral branches are again sent off. These, no doubt, have some absorbtive function similar to that of the ordinary root.

The fungus which lives in these tubercles may best be studied, in its various relations, in a median longitudinal section where the youngest stages may be found in or near the meristematic region and the older stages may be traced back toward the base of the tubercle. The infecting region is confined to the apex of the tubercle, indicating that only embryonic or comparatively young cells afford the proper conditions for the fungus. There are numberless cells adjoining, but none of them show indications of being affected. This layer is shown in f. 29. The entire infected region has the shape of a cylinder, ranging from one to two cell layers in thickness, and tapers slightly toward the apex of the tubercle where it always remains open. This region may be recognized from the place where the tubercle begins to taper. Such an arrangement seems to be indicative of the fact that the fungus has certain selective properties in determining where it may best grow in the host tissue. Another strange feature is the fact that it will live in one or two layers of cells, growing neither farther out nor in. Probably the cells have acquired some immunity after having attained a certain age, or the infected region may bear some relation to the central cylinder and also to the air outside of the tubercle and thus the fungus may have selected the most appropriate position.

The young uninfected embryonic cells are usually filled with starch grains imbedded in a spongy cytoplasm in which frequently a large vacuole is present. The starch grains are

large and simple, but occasionally compound grains may be found in the older cells. The cell nucleus is spherical in shape and relatively small compared with the size of the cell (f. 30). The chromatin stains a deep blue and the greatest number of nuclei are found in the so-called resting condition, indicating that the cells are quite active in their metabolic process.

Internal infection is accomplished by the fungus passing from cell to cell, penetrating the cell wall, and as the mycelium is very thick, frequently 1.4 to 2μ at this stage, it must secrete an enzyme which is capable of dissolving the cell walls so as to allow three or more parallel strands of hyphae to pass into a cell (f. 32). The fungus directs itself toward the cell nucleus, in the neighborhood of which it must derive its greatest benefit. Subsequent to this stage, the mycelium grows very rapidly and begins to branch and coil itself on one side of the nucleus, which is frequently crowded to the wall; occasionally, however, instances are found where the nucleus is contained within the fungal mass.

Although this fungus is considered a parasite there are no indications of such relationship as exist between the haustorium of the mildews and the nucleus of their respective host cells where it has been shown that the nucleus becomes very irregular and surrounds closely the haustorium, in which condition both nucleus and cytoplasm are ultimately absorbed by the fungus. Another singular feature is the fact that there is no apparent hypertrophy of the host cell, such as occurs so commonly among cells infected by the rusts. Apparently the host cell of *Myrica* affords less resistance to its fungus than the host cells of various plants offer to their respective mildews and rusts; even the host cells of *Ceanothus* and *Elaeagnus* display a greater resistive power.

The host cell undergoes but few changes after the fungus makes its entrance. First the starch grains, which are used as food, disappear, the cytoplasm of the host cell, which at first increases, gradually becomes less dense and finally vanishes (f. 33, 37). Comparative measurements were made of the nuclei and cells of infected and neighboring uninfected regions, and it was found that there is no increase in

size due to infection. Thus there is no expression of such a symbiotic relationship as is found in so many of the other plants, but the condition is rather one of real parasitism, where the fungus obtains the full benefit from the cells in which it lives. However, the host nucleus offers a great resistance, retaining its natural content and form for a long time. Even in some very old infected cells the nucleus may be found in nearly perfect condition; although ultimately the nuclei begin to collapse and are found as small shriveled bodies with the dead mycelium. The cell wall remains quite firm, being held in position, to some extent, by the adjoining rigid walls of other cells.

Passing farther toward the base of a tubercle, the cells are found filled with the fungus. A minute study reveals that the mycelium is composed of dense granular protoplasm in which are imbedded many nuclei of considerable size (f. 34). No cross walls can be distinguished. After a cell becomes partly filled with the mycelium, branches are sent out radially toward the periphery of the cell, which at first are narrow and oblong, but finally swell at the ends forming club or wedge shaped structures as seen in a median section (f. 35, 36). The largest ones are 3.5 to 4μ across at the widest part and usually 7 to 10μ long. For a considerable distance back from the apex the vesicles and hyphae are filled with a dense granular protoplasm in which may be found a few nuclei, while in older stages the hyphae and vesicles are empty. Thus the fungus dies out gradually, death being due to lack of food material after this has all been absorbed.

The question arises whether the plant, in an indirect way, derives a benefit from having the fungus living in its tissues. From all appearances no specific injury can be noted. It may be that the fungus can use and change the protoplasm of the host cell in such a manner that it can be taken up again from the fungus by the adjoining uninfected cells; or the conditions may be comparable to those which Hiltner (17) finds in *Lolium temulentum,* where the fungus utilizes the free nitrogen of the air which in turn is taken up by the plant. The position which the fungus selects in the

tubercle is indicative of some relationship with the atmosphere, but this fact can only be proved by accurate experiments.

It is somewhat difficult to place this fungus systematically when one can judge its morphological characteristics only from the form found within the host cells. Harshberger (15) favors the view of locating it among the Oomycetes, but judging from the characteristics as noted, it cannot properly be considered to belong to that group. Shibata (38) is probably correct in placing it with *Actinomyces* as defined by Migula in his System of Bacteriology. Pecklo (29), in his pure culture of endotrophic mycorrhiza, claims to have isolated an *Actinomyces*-like fungus from the tubercles of *Myrica Gale,* apparently the only instance of actinomycosis that has ever been reported amongst plants. In the 302 references on *Actinomyces,* given in Kolle and Wassermann's Handbuch der pathogenen Mikro-organismen (19), no mention is made of such organisms infecting plant cells, yet a large number of these pathogenic forms, in the early stage of their life history, live in the intercellular spaces of various plant tissues and several investigators have obtained pure culture of *Actinomyces* from awns of barley and grasses, so that it is not altogether improbable that a similar form may inhabit the root tubercles of *Myrica.*

COMPARISON AND DISCUSSION.

Although the tubercles and fungus on *Ceanothus, Elaeagnus* and *Myrica* have been considered the same or quite similar by several investigators, a comparative study shows that a number of variations may be noted in the fungus as well as the structure which it produces. It may be noted that the tubercles on the above plants have very little in common with those of bacterial origin found on the Leguminosae.

Differences regarding the number and location of the tubercles as found on these plants may be noted as follows: On the roots of *Elaeagnus* and *Myrica* they are not so plentiful as on *Ceanothus.* On *Myrica cerifera* they occur on the short adventitious roots which grow out from the lower part

of the stem or procumbent branches. This appears to be characteristic of this species only, for the other two species possess the tubercles on ordinary roots, like *Ceanothus.* The largest and most compact masses of tubercles occur on *Elaeagnus,* yet many may be found on *Ceanothus* which have attained nearly the same size. The dense collection of tubercles of *Myrica* is due more to the intricate interlacing of the branches than to the close proximity of the individual tubercles. The most striking external difference between the tubercles of *Elaeagnus* and *Ceanothus* is their color. The former are very dark, due to the black cortical layer which peels off and is changed to a darker color, probably by the oxidizing agents of the air and soil. In *Ceanothus* the young growing tubercles are almost colorless, but later they assume a flesh color which turns still darker as they become older. In *Myrica* the color varies from glistening white growths to the brown or almost black mature structures. As to form, those of *Ceanothus* and *Elaeagnus* are quite similar. The former attain by far the greater length, but do not form so thick or irregular a growth as in *Elaeagnus.* However, there seems to be more symmetry in the growth and branching of those of *Ceanothus* than in any other type. A great deviation from those described is found in the *Myrica* forms. Their branching is far more irregular and abundant. It is difficult to compare them as to length and thickness. One peculiar feature is the elongation of the tip which forms a long slender thread in which may be found the central cylinder. No indication of such a growth is found among the other tubercles, however great a length they may attain.

All the foregoing facts may be but minor differences, due, more or less, to environmental conditions in which the plant finds itself. The more important variations may be noted in the fungus of each host with its various tissues. In the tubercles of *Elaeagnus* and *Ceanothus* the fungus shows some similarity in behavior. In both a definite region of the cortex is infected, the mode of infection does not differ very materially. One variation exists in the fact that, in *Elaeagnus,* the fungus is unable to dissolve, either directly or indirectly, portions of cell walls after it has entered and

established itself inside the cell. In *Ceanothus,* however, the growth of the cell ceases at a certain stage, but as the fungal mass enlarges, the cell walls are dissolved and thus more space is provided. Several differences may be noted in the minute structure of the fungus of each of these plants. The mycelium of that in *Ceanothus* measures from 1 to 1.4 μ in width, is septate and has branches arising at irregular intervals. That of *Elaeagnus* is very fine, being but .2 to .5 μ at its widest parts, becoming much branched and entwined. Although this is true, the vesicles produced on both are of the same size, and the breaking up of their content and its subsequent fate does not differ very much.

The nuclei of the fungus in *Elaeagnus* are very much smaller and more numerous than those found in *Ceanothus* and *Myrica,* yet they are quite distinctly differentiated from the cytoplasm with the safranin, a result difficult to obtain with that of *Ceanothus* by using similar methods. Accurate measurements could not be made because none of the nuclei are more than a small fraction of a micron in diameter.

In both forms the content of the sporanges breaks up into several segments so that frequently four or more can be seen in one plane; but in *Ceanothus* the number of segments is smaller. In both, a close resemblance may be noted to conditions which Shibata (38) points out in the fungus of the alder where a large number of segments are produced in one sporange. These facts cannot be found in the fungus of *Myrica* tubercles. No differentiation of the content in the vesicles takes place even after the nuclei have wandered into them. This and other features of this unicellular fungus indicate that it is entirely different from that in *Ceanothus* and *Elaeagnus.*

No such perfect symbiosis between the host and its fungus can be attributed to *Myrica* as has been found in *Podocarpus* and in all the other forms that have been mentioned. If the fungus were more closely associated with the exterior of the tubercle, Stahl's views, that it absorbs mineral salts and changes them into nitrogenous compounds for the use of the plant, might offer some solution of the problem involved. This may be true among the ectotrophic mycorrhiza, but in

the forms under consideration it is rather difficult to determine how closely the fungus is related to the external environment. The host and fungal relationship may be slightly symbiotic from the fact that only a few cells are infected, yet a large number of cells is produced by the fungal stimulation, this excess of growth being easily noted just at the region where internal infection takes place. No hyperchromatic cells, so common in other tubercles, are produced in *Myrica.* As has been previously stated, the host cells of the three forms studied are destroyed as a result of their association with the fungus. In *Myrica* the problem regarding the function of the fungus in the tubercle is still unsolved. In *Ceanothus* and *Elaeagnus*, on the other hand, the evidence that the fungus is digested in part by the host cell indicates that the plant may derive some benefit from the fungus. The statement is frequently met with that the fungal organism in *Elaeagnus* enables the plant to utilize the free nitrogen of the air. This must be based on an analogy with the alder, for I have found no experimental data of any investigator which will substantiate such an assertion.

Zach (55, 57) lays great stress upon the digestive activity of the host cell in the several forms which he has studied, and attributes to it functions similar to those of the phagocytes found in animals. Even though the fungus is destroyed in part by the cytoplasm of the cell, the above analogy does not explain the actual processes which take place when the fungus is destroyed.

Regarding its systematic position, the fungus in the tubercles of *Ceanothus* and *Elaeagnus* must be retained within the genus *Frankia,* respectively as *Frankia subtilis* Brunchorst, and *Frankia ceanothi* Atkinson. The fungus found in *Myrica,* as already pointed out, has but a few minor characteristics in common with the species found in *Ceanothus* and *Elaeagnus,* and should probably be placed in a separate genus. The name *Frankia Brunchorstii* serves the purpose of designating it only by a great extension of the generic characters of *Frankia,* its *Actinomyces* nature making it quite distinct from the other species.

ENZYMES IN ALNUS AND CEANOTHUS TUBERCLES.

The experiment of Shibata (38) demonstrating the presence of proteolytic enzymes in the tubercles of *Podocarpus* and to some extent in the alder, led me to carry on similar experiments with the tubercles of the alder and *Ceanothus*.

Three different kinds of extracts were made from the tubercles: in glycerin, distilled water, and 1% NaCl. solution. The best reactions were obtained with the aqueous and glycerin extracts. The NaCl. solution gave no decisive reactions, probably due to the toxic effect which this salt has upon the enzyme. Several controls were carried on with root extracts, distilled water and glycerin. To every gram of tubercle 30 grams of solution was added. This was mixed and finely crushed in a mortar and filtered after having stood for an hour. The material crushed with glycerin was left standing for ten to twelve days, after which it was filtered through fine fabric and the filtrate was diluted with four parts of distilled water. The extracts were placed in sterilized flasks and the weighed portion of blood fibrin was added after being soaked in a 1% solution of HCl. A small drop of chloroform was added to all the extracts of each flask as an antiseptic against fungi and bacteria.

Experiment I.

To 50 cc. of diluted glycerin extract of alder tubercles .25 gm. of dry fibrin was added, and it was kept at a temperature of 34° C.

1. A normal glycerin solution.
   - **(a) At end of 24 hrs.—Somewhat digested.**
   - **(b) " " " 48 " Two-thirds of fibrin digested.**
   - **(c) " " " 72 " Nearly all digested.**

2. Glycerin extract with 5 cc. of 1% HCl.
   - **(a) At end of 24 hrs.—Half of the fibrin digested, the liquid turbid with many bubbles of gas.**
   - **(b) At end of 48 hrs.—Only a few small pieces of fibrin left.**
   - **(c) " " " 72 " All the fibrin digested.**

The solution gave a good biuret test for proteids.

3. Extract which had been heated for ten minutes at 80° C.

(a) At end of 24 hrs.—No digestion, liquid clear.
(b) " " " 48 " No action, " "
(c) " " " 72 " Fibrin still unaltered.

4. The control, with glycerin and aqueous extracts of root tissue, was placed under the same conditions as the above, but no indication of digestion was shown.

Experiment II.

Distilled water extract was made of alder tubercles. To 50 cc. of extract, .25 gram of fibrin was added. Temperature 34° C.

1. A normal aqueous extract.

(a) At end of 48 hrs.—Half of fibrin digested.
(b) " " " 72 " More digested, liquid turbid.
(c) " " " 96 " All digested.

2. Extract with 5 cc. of 1% HCl.

(a) At end of 48 hrs.—Considerable digested.
(b) " " " 72 " Fibrin nearly digested, liquid turbid.
(c) " " " 96 " Fibrin all digested.

3. Extract with 5 cc. 1% $Na_2 CO_3$.

(a) At end of 48 hrs.—Liquid clear, no digestion.
(b) " " " 72 " Fibrin hardened, no digestion.
(c) " " " 96 " No digestion.

4. Extract heated to 80° C.

(a) At end of 48 hrs.—No reaction.
(b) " " " 72 " No reaction.
(c) " " " 96 " No change in fibrin.

Experiment III.

Glycerin extract of alder root tissue. To 50 cc. of extract .25 gram of fibrin was added. Temperature 34° C.

1. Normal extract.

(a) At end of 24 hrs.—No reaction.
(b) " " " 48 " Fibrin shriveled.
(c) " " " 72 " No digestion.
(d) " " " 96 " No reaction for digestion.

2. Extract with 5 cc. 1%HCl.

No change in fibrin could be noted at end of 96 hours, and the filtered liquid gave only a slight biuret test for proteids.

3. Extract with 5 cc. 1% Na_2CO_3.

No reaction for proteids at the end of 96 hours.

4. Extract heated to 80° C.

No digestion of fibrin could be noted.

The above data indicate that the greatest amount of fibrin is digested in a weak acid solution and at the higher temperature. No loss of weight of the fibrin was obtained with alkaline, heated and root tissue extracts.

Experiment IV.

An aqueous extract of alder tubercles was used to determine the amount of fibrin 50 cc. will digest in 24 hours at 20° and 43° C. The fibrin was thoroughly dried before each weighing and then soaked in 1% HCl. before it was used.

At 20° C.

Extract.	Original wt.	After 24 hrs.	Loss.
1. Normal.	.25 g.	.249 g.	.001 g.
2. With 5 cc. HCl. (1%).	.25 "	.23 "	.02 "
3. With 5 cc. Na_2CO_3 (1%).	.25 "	.25 "	No loss.
4. Heated to 80° C.	.25 "	.25 "	No loss.
At 43° C.			
1. Normal.	.25 g.	.217 g.	.033 g.
2. With 5 cc. HCl. (1%).	.25 "	.115 "	.135 "
3. With 5 cc. Na_2CO_3.	.25 "	.249 "	.001 "
4. Heated to 80° C.	.25 "	.25 "	No loss.

Experiment V.

With extract from root tissue.

At 20° C.

Extract.	Original wt.	After 24 hrs.	Loss.
1. Normal.	.25 g.	.25 g.	None.
2. With 5 cc. 1% HCl.	.25 "	.25 "	None.
3. With 5 cc. 1% Na_2CO_3.	.25 "	.25 "	None.
4. Heated to 80° C.	.25 "	.25 "	None.

Similar experiments were carried on with root extract at 43° C., but no loss of weight was obtained.

Experiment VI.

With aqueous extract from *Ceanothus* tubercles, .25 gram of fibrin was placed in 50 cc. of extract which was kept at 33° C. A drop of chloroform was added as an antiseptic.

Extract.	At end of 7 hrs.	After 24 hrs.
1. Normal.	Slight action—liquid becoming turbid.	Some digested.
2. With 5 cc. 1% HCl.	Apparent digestive action.	Fibrin becoming filled with bubbles.
3. With 5 cc. Na_2CO_3.	Liquid dark, fibrin contracted.	No action on fibrin.
4. Heated to 70° C.	No action.	A slight precipitate.

The same extract.

	At end of 48 hrs.	After 72 hrs.
1. Normal.	Fibrin digested, liquid turbid.	Half of fibrin digested.
2. With 5 cc. 1% HCl.	One-half of fibrin digested.	Only a few small pieces left.
3. With 5 cc. 1% Na_2CO_3.	No digestion.	No digestion.
4. Heated to 70° C.	No action.	No action.

After 48 hours of digestion some filtered liquid from each flask was tested for proteid. The solutions from 1 and 2 gave good biuret tests, indicating further that normal and acid extract will digest fibrin. No proteid reaction could be obtained from 3 and 4, showing that the enzyme was destroyed under the conditions or else its action was inhibited. After six days all the fibrin in 1 and 2 was digested.

Experiment VII.

To 50 ccc. of dilute glycerin extract of root tissue of *Ceanothus* .25 gram of fibrin was added. A drop of chloroform was added as an antiseptic. Temperature was 23° C.

Extract.	After 7 hrs.	24 hrs.	72 hrs.
1. Normal.	Liquid clear.	No digestion.	No digestion.
2. With 5 cc. 1% HCl.	No digestion.	No digestion.	No digestion.
3. With 5cc. 1% Na_2CO_3.	No digestion.	No digestion.	No digestion.
4. Heated to 80° C.	No digestion.	No digestion.	No digestion.

Judging from these results no enzyme which will digest fibrin is present in the root tissue of *Ceanothus*.

Experiment VIII.

To 50 cc. of dilute glycerin extract of *Ceanothus* tubercles .25 gram of fibrin was added. A drop of chloroform was added to each flask as an antiseptic. The temperature was 22°–23° C.

Extract.	After 14 hrs.	After 24 hrs.
1. Normal.	No action, fibrin very loose.	No digestion.
2. With 5 cc. 1% HCl.	No digestion.	Slight digestion.
3. With 5 cc. 1% Na_2CO_3.	No digestion.	No change of fibrin.
4. Heated to 80° C.	A slight precipitate.	No digestion.

Extract.	After 48 hrs.	After 72 hrs.
1. Normal.	Some digestion.	Considerable digestion.
2. With 5 cc. 1% HCl.	Liquid turbid.	All digested.
3. With 5 cc. 1% Na_2CO_3.	No change of fibrin.	No reaction.
4. Heated to 80° C.	No digestion.	No reaction.

Extract.	After 96 hours.
1. Normal.	Only a few pieces undigested.
2. With 5 cc. 1% HCl.	All digested.
3. With 5 cc. Na_2CO_3.	No reaction.
4. Heated to 80° C.	No reaction.

Two other experiments with a similar extract were carried on at 33° and 43° C. At 33° C. the results were similar to those which were obtained at 23° C., whereas at 43° C. the digestive activity was much slower. Hence the optimum temperature for the enzyme is lower than 43° C. At 23° C. there is no perceptible digestive action during the first thirty-six hours, but following this period, the process goes on very rapidly and in the normal and acid extract the fibrin is readily digested. No fibrin was digested in the alkaline extract or in that which was heated to 80° C. Even 60° will stop the action of the enzyme.

If my interpretation of the preceding data be correct there is present, in the tubercles of the alder and *Ceanothus*, an enzyme capable of digesting fibrin. The enzyme obtained from the *Ceanothus* tubercles is more active at a lower temperature than that from the alder which digests more readily at a higher temperature. The enzyme, however, is found

only in the tubercles, for experiments with root tissues show that it is not present in the normal root.

The question still presents itself whether the enzyme is produced by the host cell or by the fungus. Judging from cytological data, the digesting of the fungus is an indication that the host cell produces an enzyme. The dissolving of the cell walls in the tubercles of *Ceanothus* presents a fact which indicates that the fungus also produces an enzyme. Thus there may be two enzymes present, one produced by the host cell and another by the fungus, for it is hardly probable that the host cell forms an enzyme which dissolves its own walls. Until a sufficient amount of pure culture of the fungus can be grown, it is impossible to decide whether the fungus secretes an enzyme or not, but the conditions are probably quite similar to those which Marshall Ward found in *Botrytis,* which produces cytase capable of dissolving the walls of the host cell.

SUMMARY OF RESULTS.

The Tubercles of *Ceanothus.*

1. Judging from the common occurrence of the tubercles, the infection of this plant by the fungus is quite universal. 2. External infection probably takes place through a root hair or an epidermal cell from which the subsequent tubercle is formed. 3. The tubercle consists of three systems of tissues: the outer or corky layer; the inner, the vascular cylinder; and the middle or cortex, which contains the infected cells. 4. Internal infection occurs in the growing region and takes place by the fungus passing from cell to cell. 5. Three distinct stages of fungal development may be noted: the mycelia stage found in the host cell; the stage with the sporanges, which initiates the conditions for the digestive cell; and the last stage, where all but the walls of the mycelium are absorbed. 6. Because of infection, hypertrophied cells and nuclei are formed. The fungus dissolves the walls of the host cell. 7. The host nucleus increases in volume; with it, there is an increase of the nucleole and in the amount of chromatin. 8. Following the vesicular stage the cytoplasm and nucleus of the host cell are absorbed. Subsequent to this, the cell content of the fungus disappears. 9. Both the host cell and the fungus finally die and undissolved portions of the fungus remain in the cell. 10. Symbiosis exists, which is quite apparent in the early stage.

Elaeagnus.

11. The tubercles are not found as abundantly as on *Ceanothus*. Regarding the form and structure, several resemblances can be noted. 12. External and internal infection takes place as in *Ceanothus*. 13. The fungal mycelium differs from that of *Ceanothus* in being very narrow. It branches profusely, forms the vesicles, the content of which breaks up into several segments. The infected cell passes through various stages. The fungus is not entirely absorbed by the digestive cell. 14. The walls of the host cell are not broken down as a result of the fungal infection. 15. Hypertrophied cells and nuclei are formed, but the nucleo-cytoplasmic relationship is maintained in the infected cells. 16. No "Exkretkörperchen," such as Zach reports, can be found in the digested cells. 17. Both the host cells and the fungus die as a result of their previous relationship.

Myrica.

18. The tubercles and fungus of *Myrica* differ in many respects from those of *Ceanothus* and *Elaeagnus*. All species of *Myrica* possess tubercles. 19. The fungus confines itself to one or two layers of cells and internal infection takes place acropetally. No hypertrophy or symbiotic relationship exists. The fungus is best regarded as a parasite. 20. The unicellular hyphae of the fungus form branches which change to club-shaped structures in which no further differentiation takes place. 21. The fate of the host cell and fungus is similar to that in *Ceanothus*. 22. The form, structure and behavior of the fungus indicate that it belongs to the genus *Actinomyces*.

Enzymes.

23. In the tubercles of the alder and *Ceanothus* enzymes are present capable of digesting fibrin. Whether two enzymes are present, one produced by the host and another by the fungus, could not be determined without a pure culture of the fungus.

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EXPLANATION OF PLATES.

All of the figures were drawn with the aid of a camera lucida. A Leitz 1/12 oil immersion objective and ocular no. 4, giving a magnification of 1350 diameters, were used for all except figures 1, 14, and 29, for which use was made of a no. 3 objective and no. 1 ocular, giving a magnification of 85 diameters.

Plate 6.—A portion of a *Ceanothus* root with many young tubercles on the small lateral roots, above. Part of a *Ceanothus* root on which older tubercles are formed into loose clusters, below.

Plate 7.—A large *Elaeagnus* root showing the dense mass of tubercles attached to it by a short branch, above. A portion of a root with large clusters, below.

Plate 8.—Part of a stem of *Myrica cerifera* showing the masses formed at the ends of short adventitious roots.

Plates 9–10.—*Ceanothus americanus.* 1, Transverse section of a root tubercle of *Ceanothus* indicating the infected region and some of the fungal stages. 2, Cells of the meristematic region showing early stages of infection. One cell shows where a hypha is just entering. The cell wall between some of the cells is being dissolved. 3, An embryonic cell just infected showing the branched mycelium. 4, An older hypertrophied host cell with enlarged nucleus. The mycelium is much branched and entwined. No vesicles have yet been formed; portions of the cell walls are being dissolved. 5, 6, Nuclei of the fungus set forth by the haematoxylin stain. The hyphal walls are difficult to differentiate. 7, A stage where the sporanges are formed at the end of the hyphal branches. Infection of adjoining cells is also shown. 8, Hypertrophied nuclei of the host cell differing from the following. 9, Nuclei of digestive cells similar to the one shown in f. 7. 10, Young sporanges showing their content. 11, Older and mature vesicles with a single nucleus. 12, Sporanges burst open, the content has disappeared. 13, A cell showing the last stage of the fungus where all but the walls of the mycelium is absorbed.

Plates 10–12.—*Elaeagnus argentea.* 14, Cross section showing the infected region of the tubercle and its various tissues. The large dense cells contain the fungus in the vesicular stage,—other cells show younger stages. 15, Two uninfected cells showing nucleus, large starch grains and fine granular cytoplasm. 16, An infected cell showing hypertrophied nucleus, the mass of mycelial threads and the mode of infection. 17, 18, Infected cells where the hyphae pass through the cell wall into adjoining cells. 19, A large hypertrophied cell with an amoeboid nucleus. The fungus has the sporanges, in

which the content is broken up into parts. 20, 21, The same vesicles drawn on a larger scale. 22, A stage where the fungus is partially destroyed, the walls of the vesicle and hyphae remaining. 23, A host cell which has collapsed in which the remains of the fungus are still present. 24, Nuclei of the host cell before they become amoeboid in form. 25, Stages of nuclei found in the digestive cells. 26, Very late stages of degeneration of nuclei just before their final disappearance.

Plates 13, 14.—*Myrica cerifera.* 28, Life-sized tubercles as they are found in the clusters. 29, A longitudinal section of a tubercle showing the infected region and the various tissues of the tubercle. 30, Several uninfected cells showing the cell content. 31, Cells indicating the method by which internal infection takes place. 32, Cells showing the large number of hyphae which pass through the walls to infect the cell. 33, Several cells of the infected region showing young and old stages of the fungus where the branches of the hyphae have enlarged into club-shaped structures. 34, A portion of mycelial thread showing the nuclei. 35, The club-shaped ends of the hyphae. 36, The same but older structures where the nuclei have passed into them from the mycelium. 37, A stage where the host cell and the nucleus begin to disintegrate. The fungus also shows similar stages. 38, Several degenerating nuclei found in host cells.

CEANOTHUS.

ELAEAGNUS.

MYRICA.

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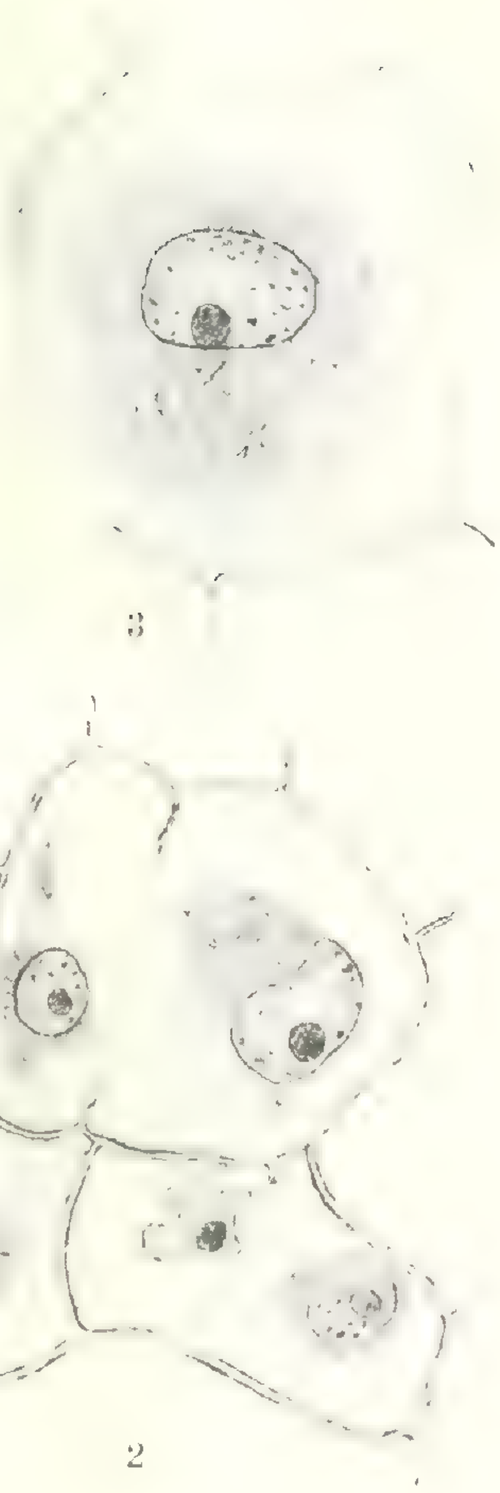

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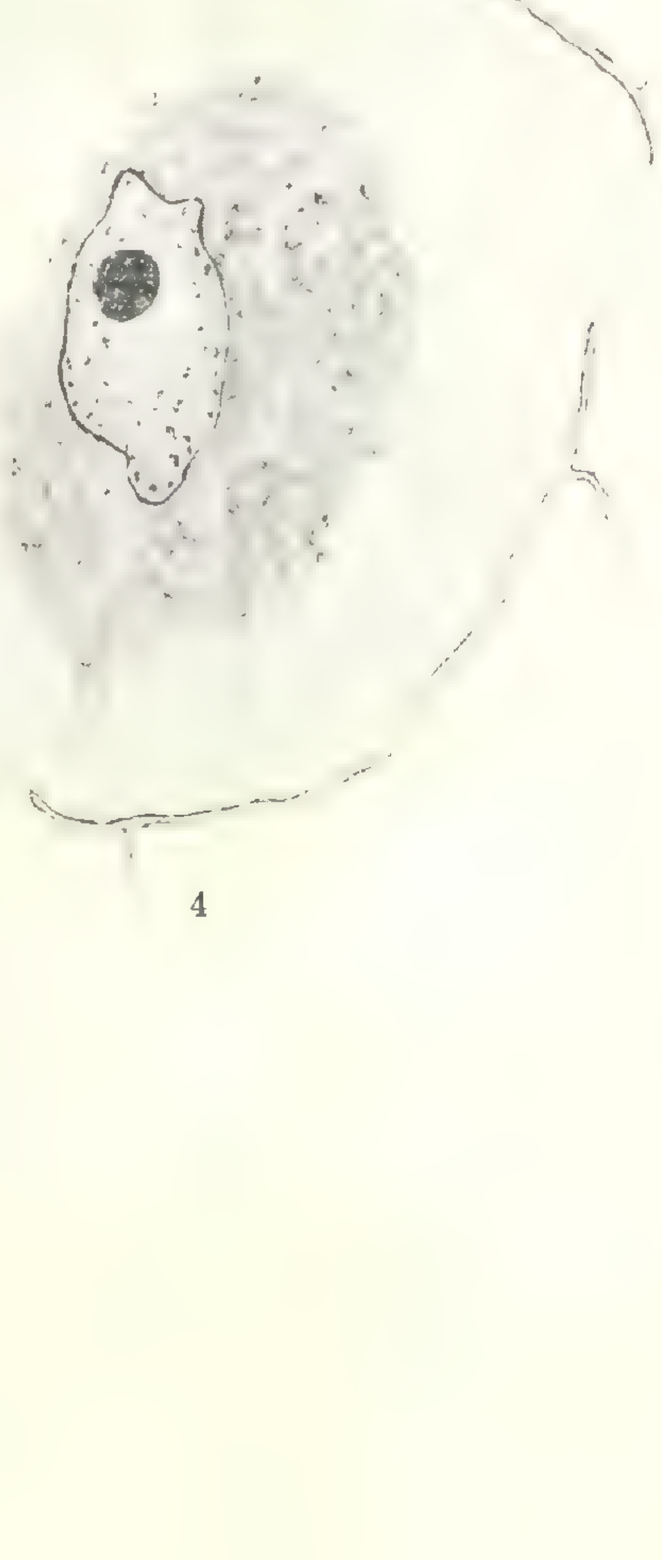

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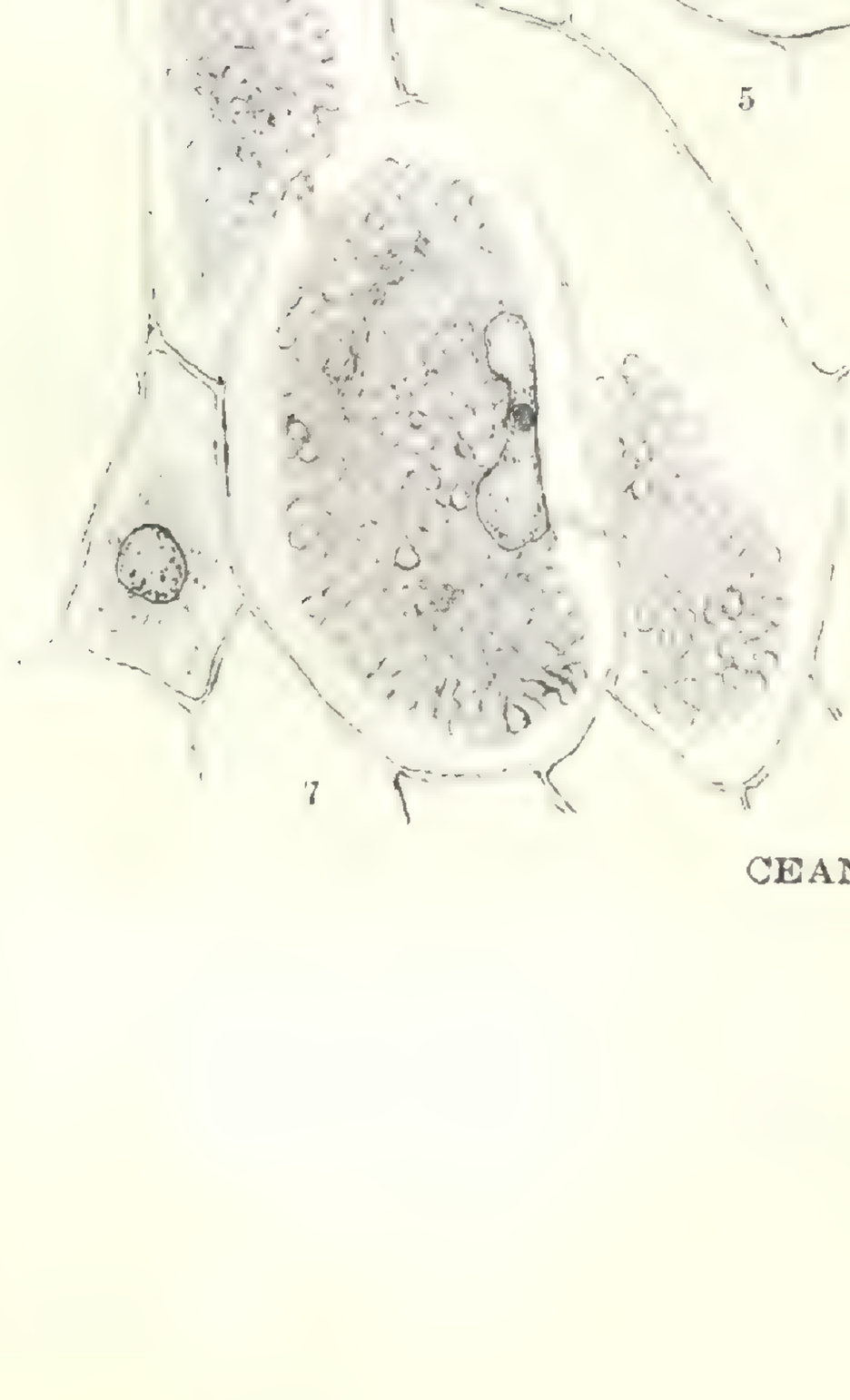

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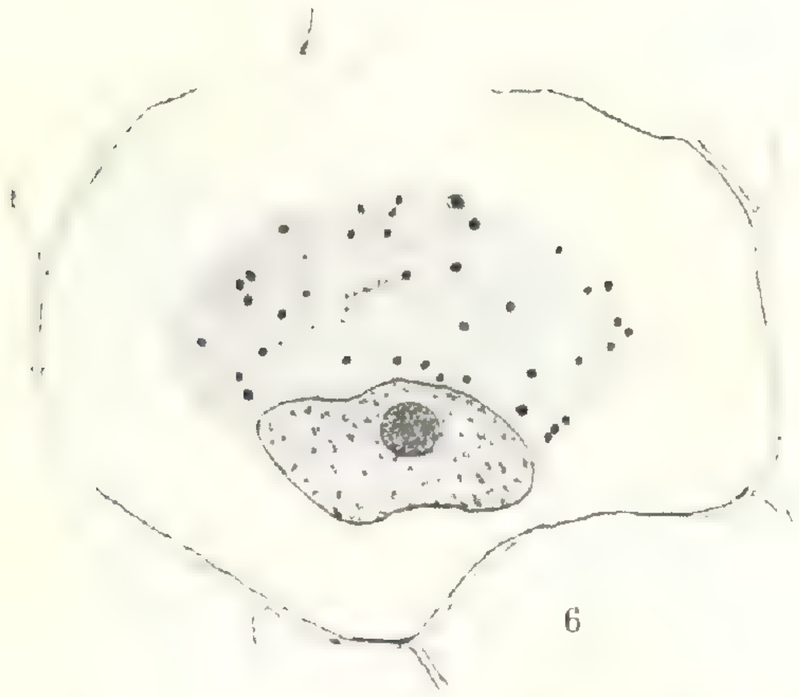

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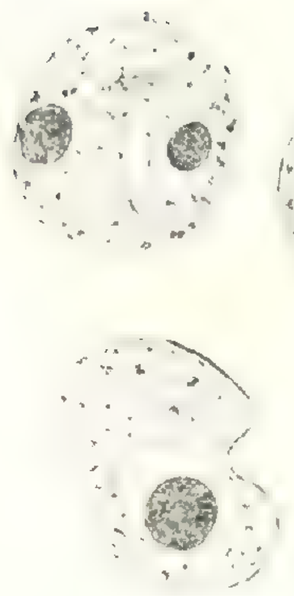

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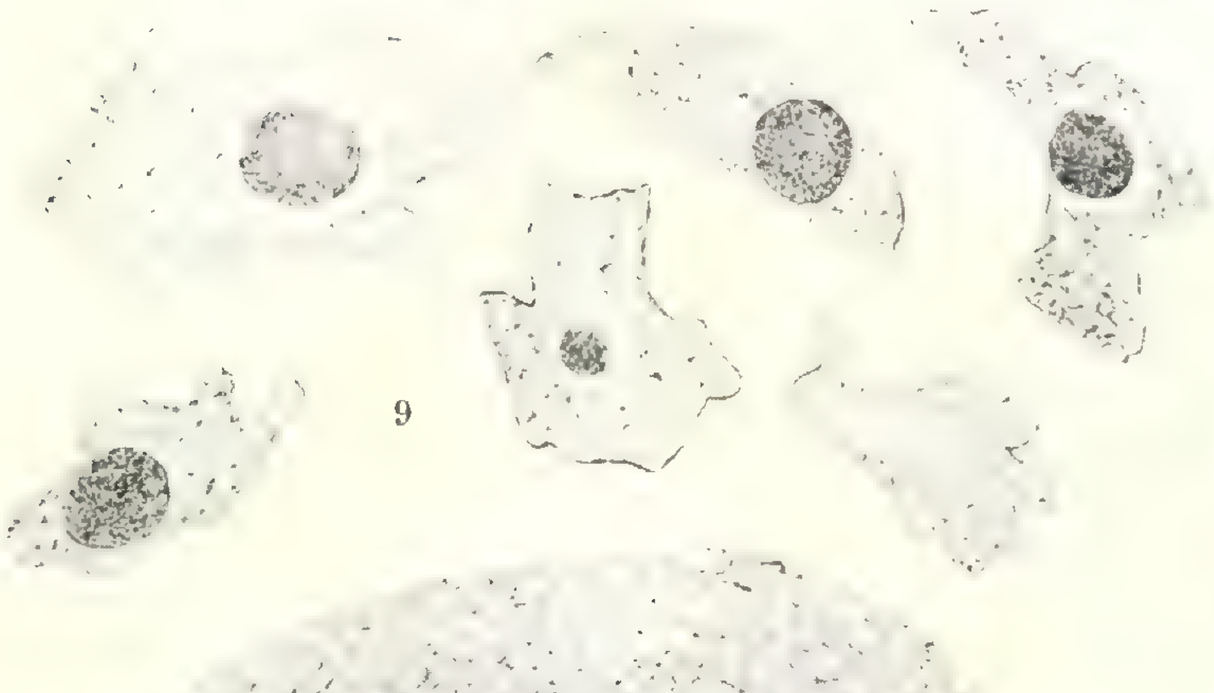

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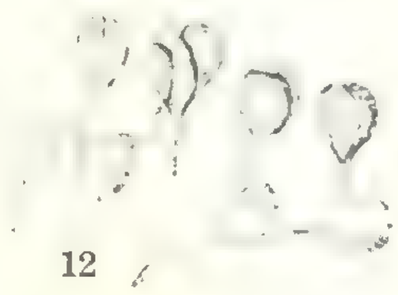

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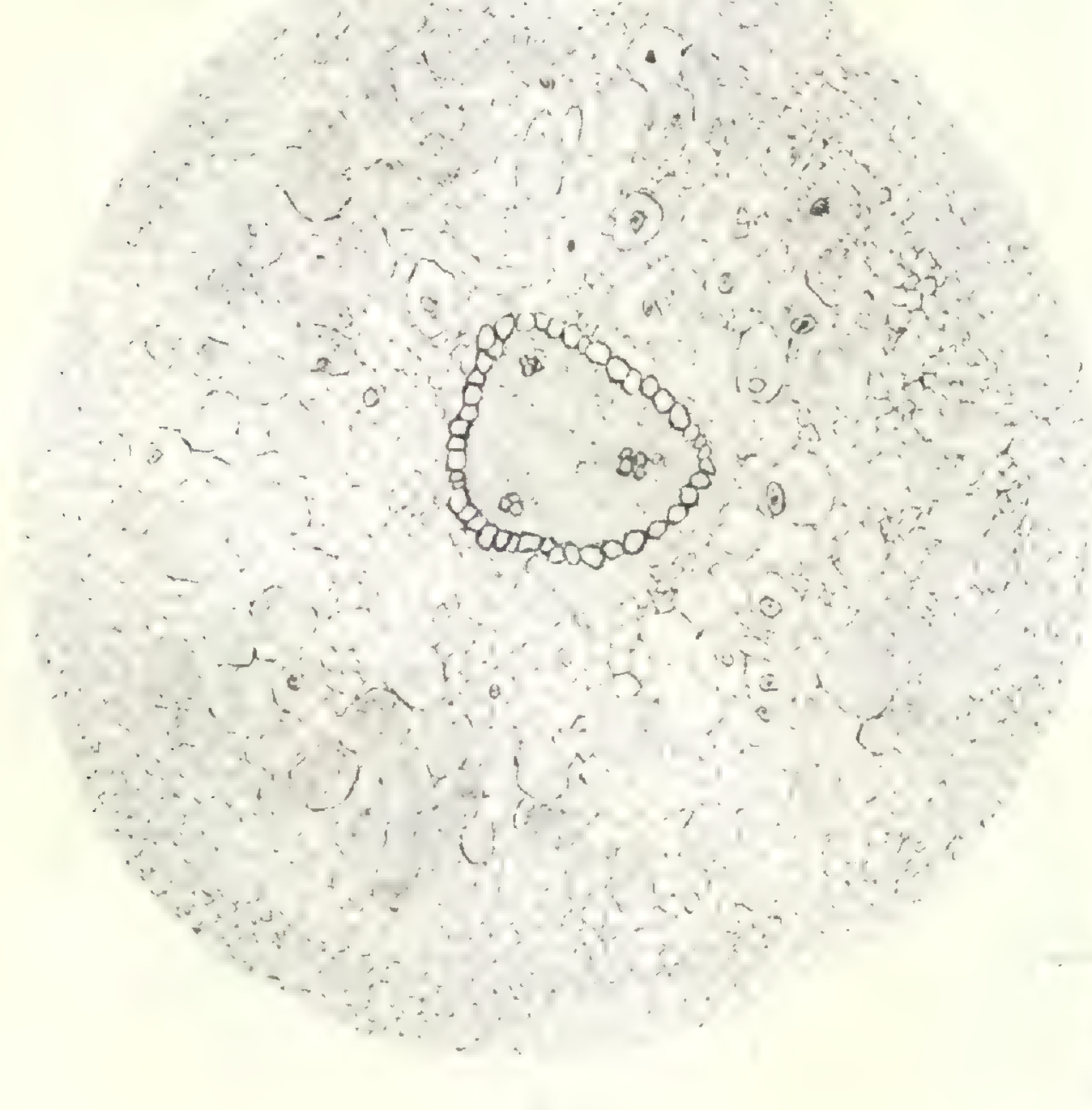

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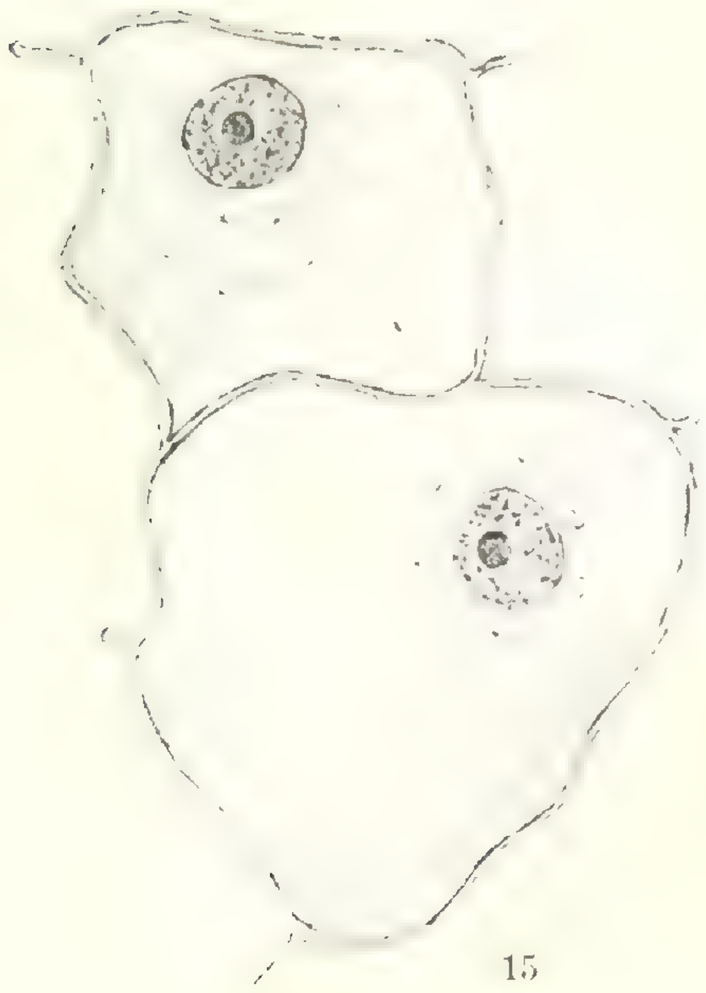

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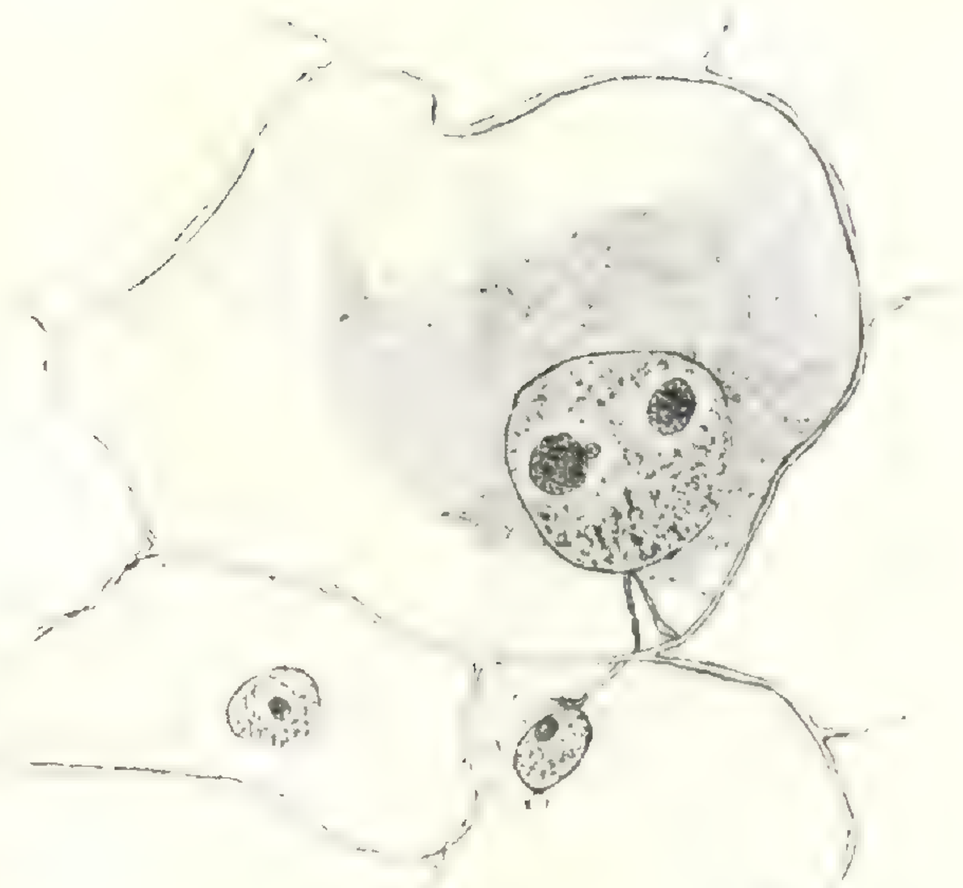

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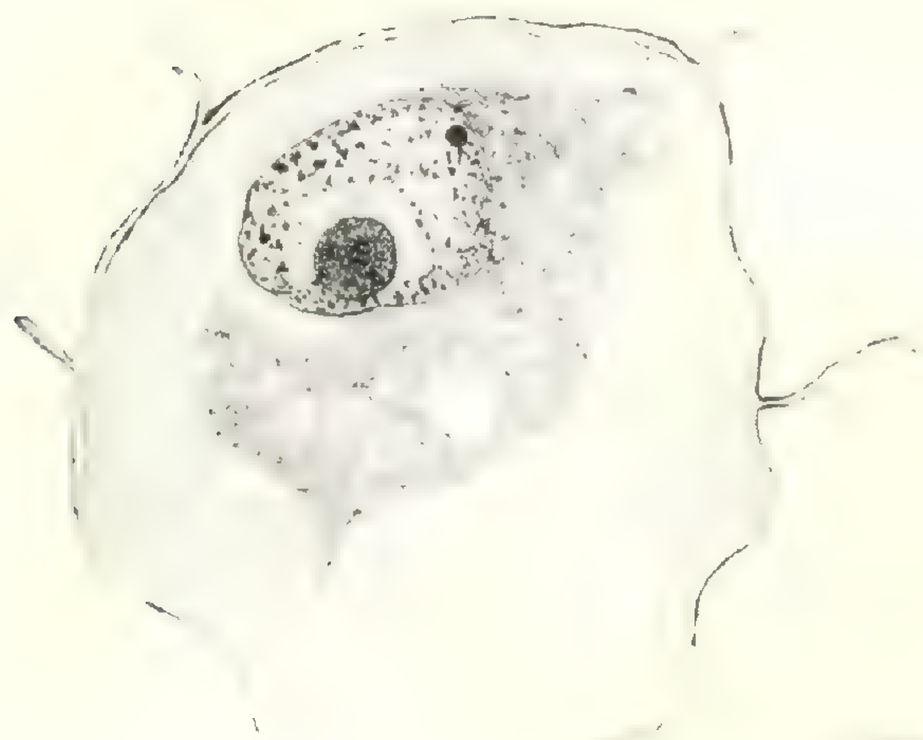

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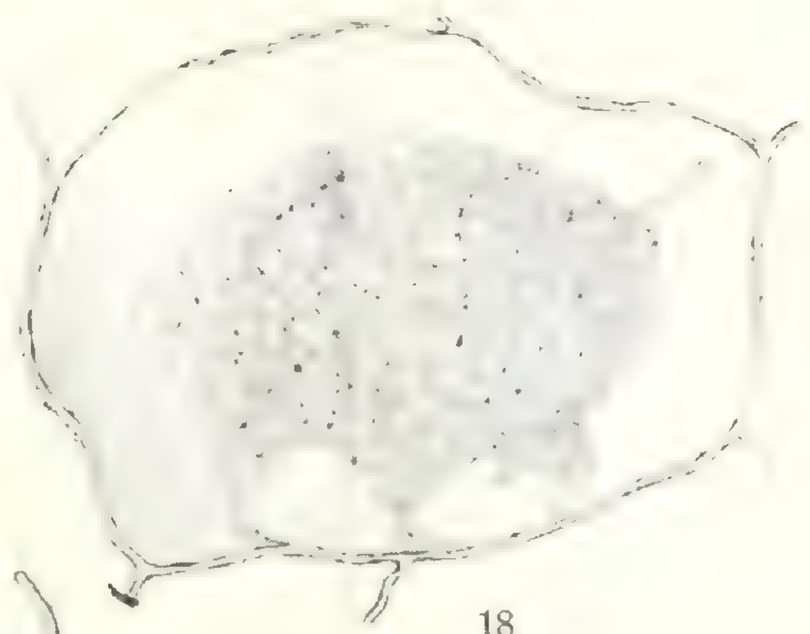

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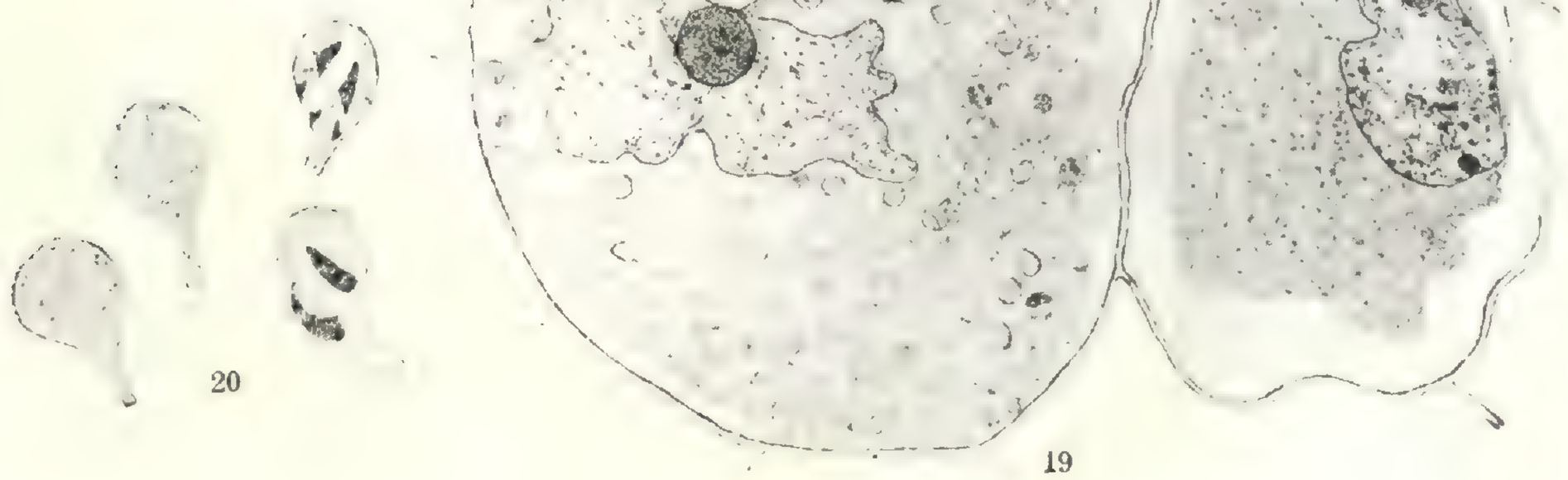

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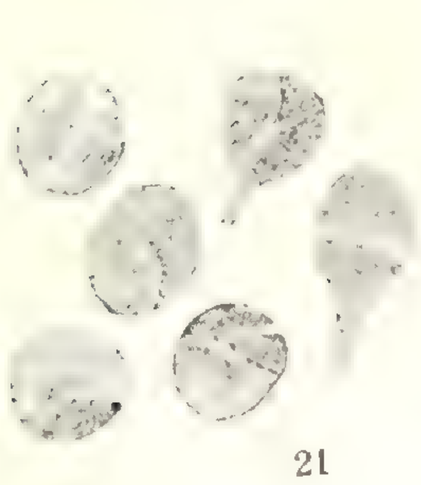

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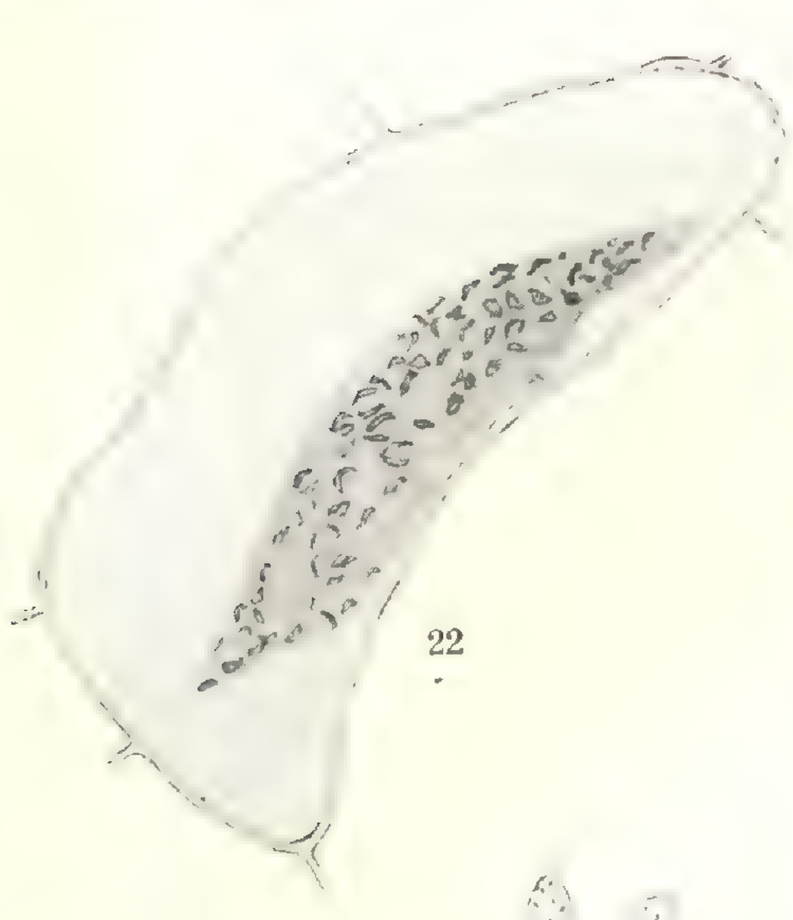

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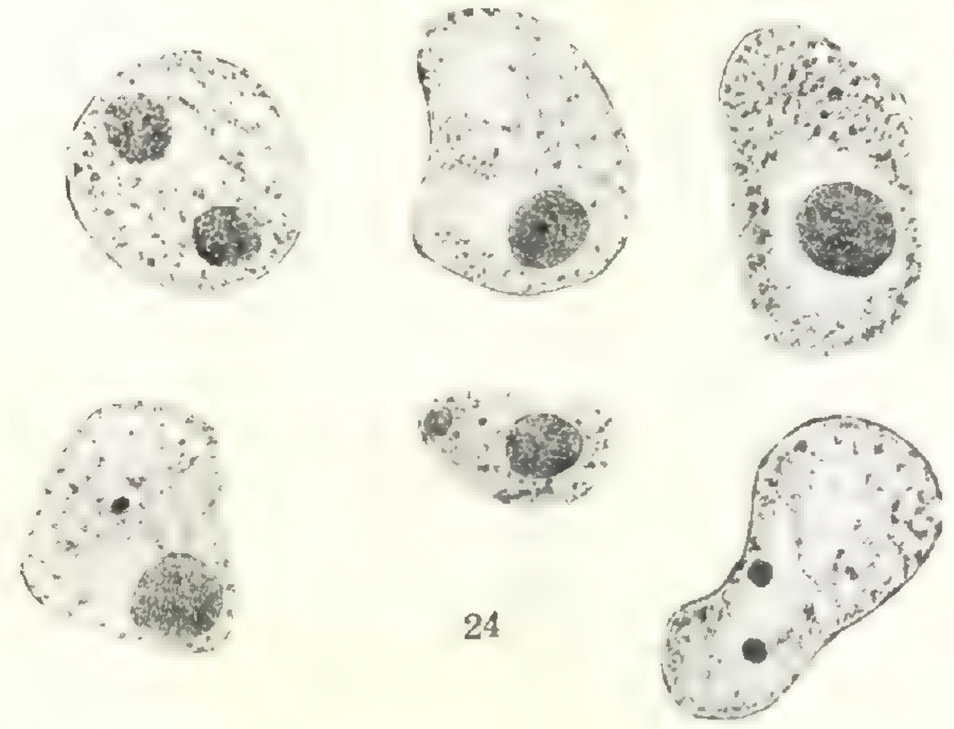

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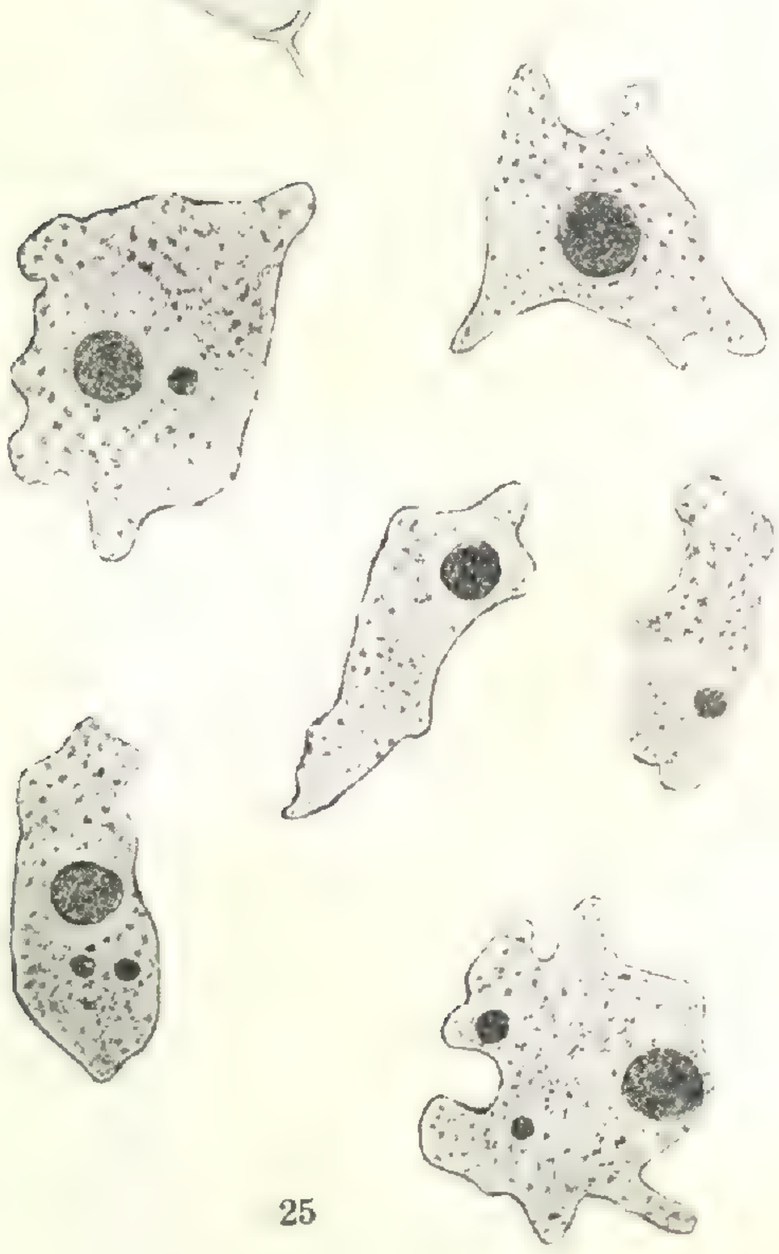

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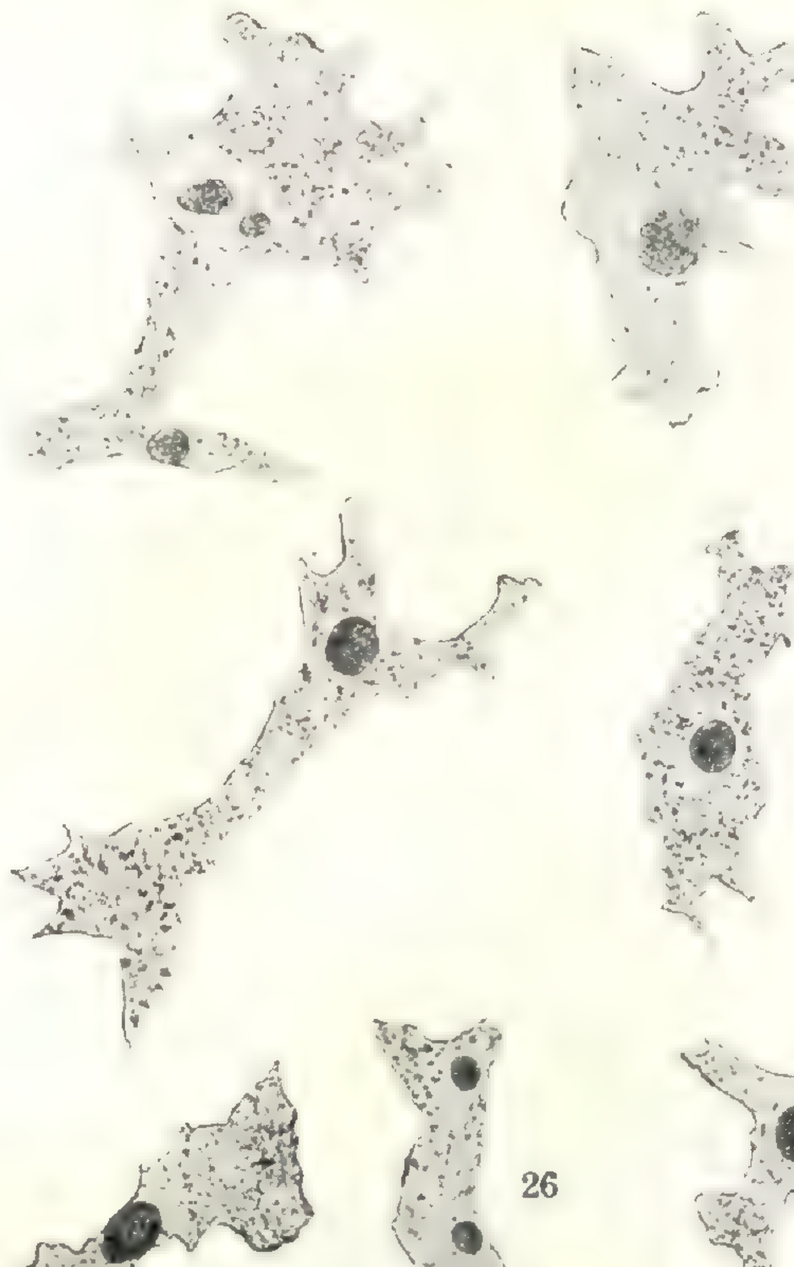

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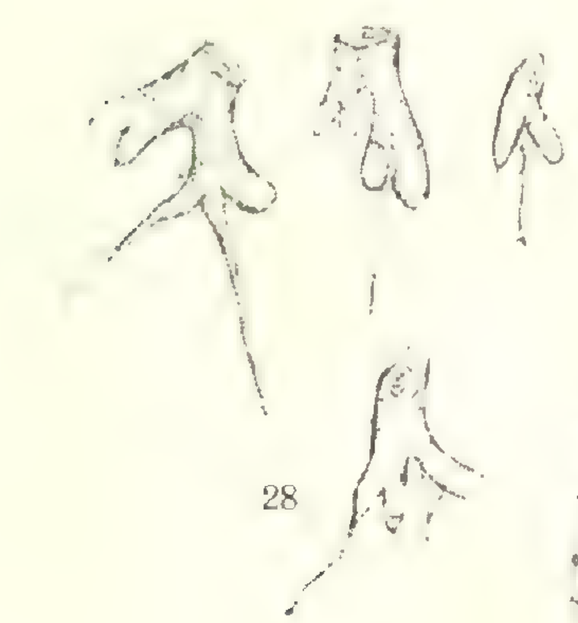

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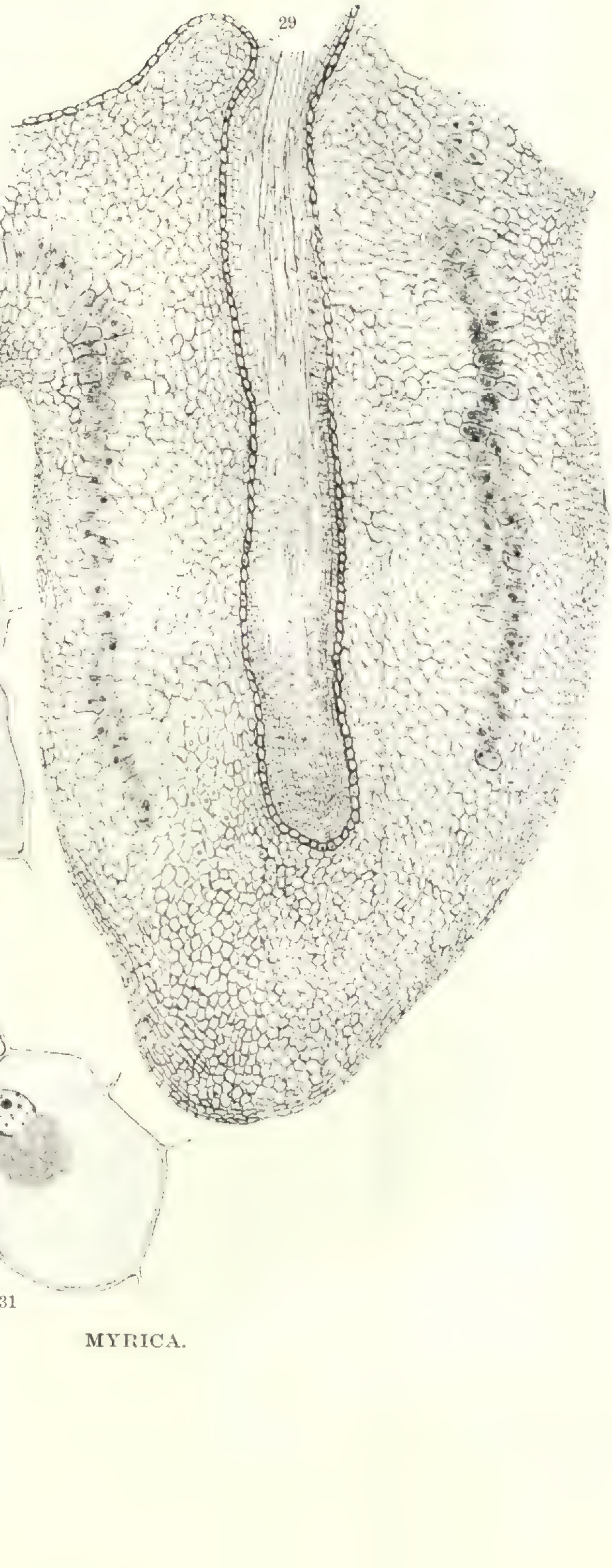

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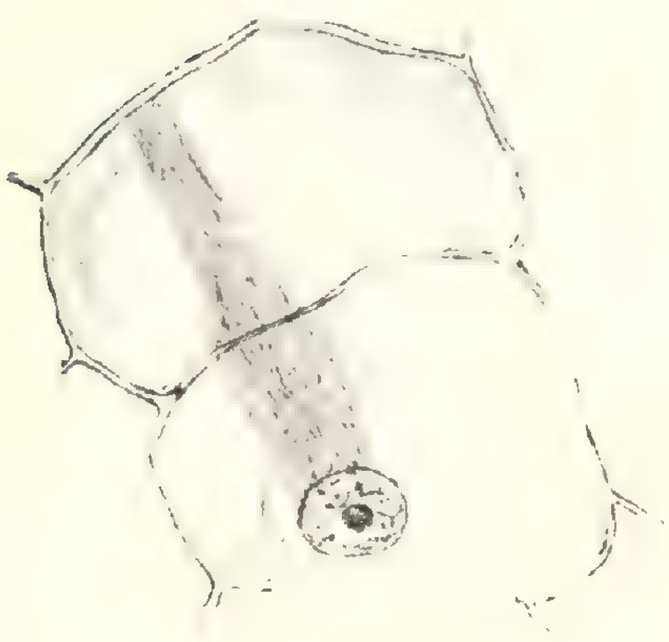
MYRICA.



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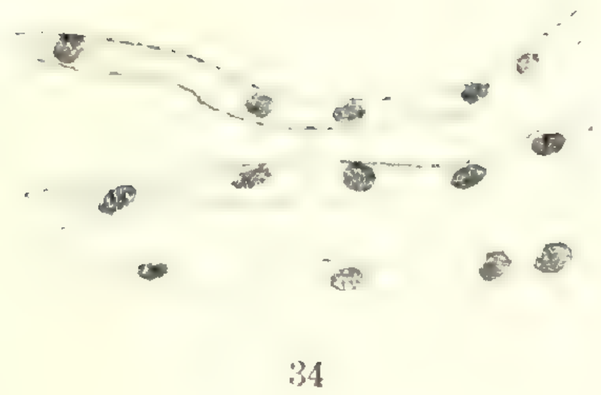

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MYRICA.

DEVELOPMENT AND NUTRITION OF THE EMBRYO, SEED AND CARPEL IN THE DATE, PHOENIX DACTYLIFERA L.

BY FRANCIS E. LLOYD.

PURPOSE AND SCOPE OF THE STUDY.

The work here reported was begun in 1907 upon my appointment as Cytologist to the Agricultural Experiment Station, Tucson, Ariz., in connection with the special investigations on the date which have there been carried forward during recent years, more especially by Dr. A. E. Vinson. The material was collected, through the co-operation of Dr. Vinson, at the Station Date Orchard, at Tempe, Ariz. At the inception of the work it was my purpose to study exhaustively the whole period of embryogeny with reference to the rôle of the various foods and other materials in the seed and carpel, and for this purpose, it would have been necessary to make use of both preserved and fresh material. This object was defeated by my removal to Mexico, and I was therefore compelled to make use exclusively of preserved material, with the exception of some of the earlier stages which I studied before leaving Tucson. In consequence, the sugars have, I regret, been left out of account. This is of less consequence as regards the carpel, as they have been studied, by the methods of the chemical laboratory however, by Dr. Vinson, who has embodied his results in various papers to be later referred to. The present paper records my studies therefore of the anatomy and histology of both seed and carpel from the developmental point of view, and of the rôles of tannin, starch, oil and reserve cellulose. Material of two well marked races, Rhars and Deglet Noor, invert and cane sugar types,[1] respectively, has been examined, but it has developed that such differences as exist are, from the present point of view, negligible. When desirable, I have noted such differences.

[1] Vinson, 1906.

The date seed has long furnished material for studies begun with Malpighi, later continued by Sachs, whose work is fundamental, and followed by several others whose work will be mentioned beyond. This has been due in part to the ease of obtaining material and the conspicuousness of the phenomena during germination. In the development of our knowledge of enzymes it has played an important part, not the least important contributions in this field having been made by Americans. The positive evidence thus gained is of value not only in interpreting what goes forward during germination, but also during embryogeny. Working backward from the resting period, at which point the studies available up to the present time have begun, we are able to understand many details for which otherwise only speculative explanation could be advanced.

Methods.

Material was gathered on alternate weeks, from the time of pollination till maturity, from one of the two races and preserved in three series, one in a watery solution of copper acetate, as recommended by Strasburger, one in chrom-acetic fluid, followed by alcohol, the third in alcohol-acetic, (2:1). For examination, free-hand sections were used, except in earlier stages, in which it was necessary to use microtome methods for the determination of minutiae of structure. Free-hand sections were treated with iron salts, (ferric acetate or chlorid).

After long standing in copper acetate, the material contained, in many cases, a considerable amount of metallic copper, either within the tissues, on its surface or in the fluid. This was evidently due to the reduction of the copper salt, chiefly, it seems probable, by the invert sugars present. The possible source of error, due to the presence of tannin, was excluded by the use of ethyl nitrite, on a small but satisfactory series of material prepared for me during the spring of 1910 by Dr. Vinson at my request. This check material showed that my observations on the copper acetate preparations were correct. This is of considerable impor-

tance during the development of the endosperm, where the fixation seemed to lead to misinterpretation.

Similarly the long exposure to copper acetate of material containing oil results in the formation of a white, apparently amorphous deposit in certain situations. In a few preparations, under conditions which I am unable to determine exactly, beautiful dendritic masses of minute crystals have appeared. In others, large numbers of pale green sphaerocrystals have been found on cutting a section, which had the appearance of being an oil-copper compound. Their insolubility in suitable solvents seems to preclude this interpretation. Nevertheless, these should be understood in order to exclude completely sources of error, as it is not at all unlikely that where oil is exposed to copper salts, the oil would be reduced in amount. In the present case, the alcoholic material was used as control.

I am obliged to Professor R. H. Forbes and Dr. A. E. Vinson for much assistance in the obtaining and preservation of material, and to my colleagues, Professor B. B. Ross and Professor C. L. Hare, for criticisms from the chemical point of view of certain interpretations. This study, begun at the Arizona Agricultural Experiment Station, has been largely prosecuted at the Alabama Agricultural Experiment Station.

STRUCTURE AND DEVELOPMENT.

Organogeny of the fruit. Some account of the development of the parts of the fruit will be necessary in order to make evident the anatomical and histological changes which take place during the time between pollination and the final maturation of the fruit. The materials have been examined, not with the object of studying the cell-to-cell minutiae of the embryology, but rather to follow the main outlines of development of the embryo, seed and fruit, together with their nutritive inter-relations discoverable with the methods at hand.

The whole extent of the development of the date fruit falls rather naturally into three periods: a, that extending from the time of pollination (Stage I, f. 2) until the endo-

sperm ends its pavement phase of development (Stage II, f. 7); b, that between this time, and the final closure of the endosperm cavity (Stage III, f. 14); and c, the period following until maturation (Stage IV). It will be convenient for cross reference to speak of these stages and periods. Period I occupies about eight weeks. The young seed is then 4 mm. long by 2 mm. in diameter. The whole fruit measures 7.5 by 7 mm. (broad). Period II occupies about three to four weeks. The fruit is 9.5 mm. broad by 10 mm. long; the seed 7 mm. long by 2.5-3 mm. broad. It is relatively a very short period and one of rapid change, characterized by the development of the endosperm, and marked topographic changes in the ovule in general. The third period occupies a period, following the closure of the endosperm cavity, covering about 15 weeks, and is characterized by the development of the embryo, which, until the end of period II, remains very small.

Stage I. Beginning of the First Period.

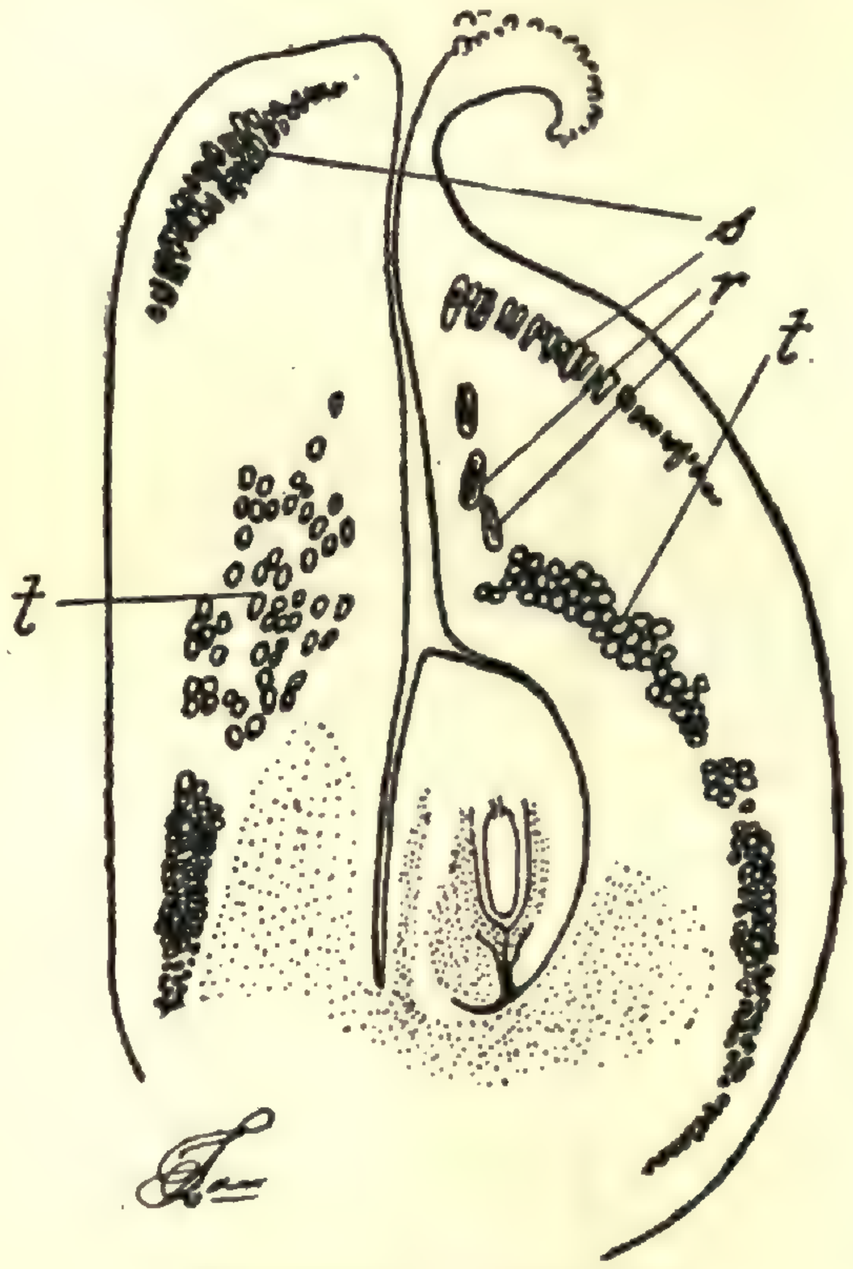
The pistil (f. A), with the exception of the stigma, is enclosed in the involucre. Three pistils are present, only one of which persists normally.

Carpel. The whole pistil at the time of pollination measures about 3 mm. in length by 1.8 mm. broad. The dorsiventral diameter (1.2-1.4 mm.) is somewhat less. The locule is small, and completely filled by the anatropous, central basal ovule. The short style is traversed by a canal lined by secretory cells—pollen tube guiding tissue. The canal is continuous with a groove, which at the upper part of the ovule is single, but bifurcates as it passes downward, one groove passing on each side of the funicle (f. 2-2e). In front of the micropyle the glandular tissue spreads out to form a cap over the exostome (f. 2). These grooves persist and enlarge, and may be traced (f. 11) throughout the whole development of the fruit.

At this time, with the exception of the epidermis of columnar cells, external and internal (endocarp), there is to be recognized only the tanniferous layer of idioplasts. Within

a week after pollination, active differentiation of tissues has commenced at the apex of the carpel, which begins to project beyond the bracts. The epidermis is thus more or less exposed and is becoming strongly cuticularized. Hypodermal tannin cells are evident here and there, and the layer of stone cells is rapidly forming. Within the mesocarp, numerous raphide bearing cells have been formed, and the tannin-idioplasts are more strongly developed.

Ovule. The ovule at the time of pollination presents several matters of interest from the present point of view. Of the nucellus nothing remains but a cap of a single layer of cells, in a state of rapid disintegration (f. 1). The bulk of the ovule is of nucellar origin (f. 2, 5), and, after the disappearance of the nucellus, may be regarded as a chalazal tissue. It thus comes about that only the upper end of the embryo-sac is surrounded by the integuments, which form a crown resting on the top of it (f. 3). The micropyle is evident, and is lined by actively glandular cells, the inner superficial cells of the inner integument. As seen by the figure, the endostome juts forward into the exostome, which is open. The anatomy of this region suggests the explanation that the glandular tissue of the carpel facing the exostome is a center of attraction for the pollen tube till it reaches this point. The secretion, which is doubtless



A. PHOENIX DACTYLIFERA.

Longitudinal section through pistil at approximately the time of pollination. The distribution of starch is shown by stippling.—*t*, Tannin idioplasts. *s*, Stone cells. *r*, Rhaphide idioplasts. The stylar canal is seen.

thrown out by the glandular cells of the endostome, then exerts a superior attraction perhaps quantitatively only, or, it may be, qualitatively, and this leads the pollen tube to enter the exostome, which is its normal course. I have elsewhere dealt with the problem of the direction of the pollen tube (Lloyd, 1902) suggesting that the pollen tube is guided in its course by the differential distribution of a stimulant arising from the egg-apparatus, chiefly the synergidae, and that the whole phenomenon is chiefly a chemical one. The evidence here before us prompts a modification of the above suggestion, which however does no violence to its fundamental feature, to the effect that the stimulant may be handled in a system of relays, the pollen tubes being guided from one relay to the other. In the date the relay stations, so to speak, are in the carpellary guiding tissue, the glandular endostome tissue and the egg-apparatus and egg cell itself. There is no objection to the assumption that these offer either a renewed stimulus of the same kind, or even a different kind of stimulus each time, in view of the work of Lidforss, who showed that positive curvatures are shown by pollen tubes toward nineteen proteins of various groups.[2] It appears not improbable that refined methods may discover that where different guiding tissues occur, each one involved produces its specific secretion which restimulates the pollen tube from time to time on its course.

The innermost layer of cells of the inner integument, already mentioned, also presents a special degree of activity. The cells become deeply columnar and distended at their free extremities where they touch the embryo-sac. This layer of cells is clearly a tapetum, and is analogous to that described for the Compositae[3] and for a number of other plants by various later authors.

This tapetum is contributed to, to some extent, by the adjacent chalazal cells, so that it extends some distance down the embryo-sac, and further down on the funicular aspect, where it reaches as far as the antipodal region. Here it has a distinctly pronounced development.

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[2] Lidforss, 1909.

[3] Goldfluss, 1898–9.

At this moment, namely at pollination, or very soon after, the antipodal end of the embryo-sac shows a remarkable amount of activity. This is seen especially in the very irregular and rapid backward extension of the endosperm, previous to the division of the endosperm nucleus, leaving the antipodal portion of the embryo-sac in its original position (f. 3, 5). There is formed in this way a curious several-armed chalazal extension which, in view of the digestion of the tissues in its path[4] must be regarded as a haustorium. Its function is the same, I believe, as that of analogous haustorial structures formed by the endosperm in *Plantago*,[5] in which, however, they are more highly specialized in form. Its total activity, when followed through the whole course of events, is relatively very great, as will appear from its position and volume in the ripened seed.[6] The observed phenomena relating to this activity are recorded beyond. At this point it is sufficient to say that, in a week after pollination, the amount of development is quite marked, there being several cul-de-sacs penetrating deeply into a tissue heavily loaded with tannin. At the same time there is evident the beginning of that torsion which ends finally in the complete displacement of the embryo (f. 5).

Stage II. Close of the First Period.

Carpel. The epidermis is strongly cutinized. The hypodermal parenchyma cells are still cubical or rounded, but show the accumulation of tannin. The layer of stone-cells is completely developed, as also the tannin-idioplast layer. Tannin-idioplasts occur also throughout the mesocarp in the sutural sector (f. A, 7, 11), and in all parts of the basal region. They are especially numerous near the funicle. Morphologically, this is placental tissue, and is constantly

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[4] At first similar in appearance to the irregular cavity formed in the pine nucellus by the growing pollen tubes.

[5] Balicka-Ivanovska, 1899.

[6] It is evident that the chalaza in the mature date seed is not primary. Its developmental continuity, however, is clear and so we may properly call it the chalaza.

characterized by scattered tannin cells. Below the insertion of the funicle, there are two longitudinal grooves, one on each side of the ovule, which may be called sutural sulci. These arise as the continuations of the stylar canal, and are earlier functional as pollen-tube guiding grooves already described. Above the funicle the sulci unite and have a common meatus, finally becoming single. The epidermal (endocarp) lining of the sulcus has the appearance of glandular tissue, and simulates the nectar grooves found in certain plants (e. g., Liliaceae). There is a greater amount of tannin in them, in common with the tissues of the placental region than elsewhere in the endocarp. There is, however, no indication of glandular activity. The sulci, as a result of growth pressures, are secondarily more or less irregular in their disposition, as is seen from the examination of a series of sections.

Raphide cells are now relatively much less numerous, though they occur in scattered positions throughout the mesocarp.

The endocarpal epidermis is so far differentiated that the cells are considerably elongated and irregular in tangential contour.

Seed (f. 7). The torsion begun during the first week (f. 5) has progressed so far that the embryo is now one-third of the entire length of the ovule distant from the original position. A corresponding amount of torsion has been experienced by the upper end of the ovule, so that the backward extension of the endosperm, beyond the chalaza, is well started. The endosperm is parietal, and of a single layer of cells. The tanniferous tissue about the chalaza is very pronounced but is not readily distinguishable on account of the general tannin reaction. The chalazal cul-de-sac has enlarged, and continues to do so beyond this period, so that in the definitive seed its size is marked.

The torsion which the integuments undergo is a differential one. The epidermal cells move relatively less, while the greatest movement is found in the innermost layer of cells. Thus it happens that the exostome of the micropyle does not

change position as much as the endostome, which retains its topographic relation to the embryo. As a condition of further growth, there is yet but little differentiation in the integuments. There is growth in thickness and extent, but the cells retain their undifferentiated character.

Stage III. Close of the Second Period.

Carpel. Aside from the extension of the tissues by growth, there is little to record, except only that, as a result of the mutual pressure of tissues, the inner zone of the carpel becomes slightly compressed. A small amount of disorganization is apparent here and there, foreshadowing the shining fibrous threads regarded as a part of the endocarp. The crushing is more apparent at the placental region than elsewhere, and it is especially noticeable in its effects upon the placental sulci, which become distorted. The contingent cells within the sulcus become denser in character and there appears as it were a mucilaginous thickening of some of the cell walls, especially near the meatus. There are numerous tannin cells of idioplastic nature, though the tannin is not wholly confined to these.

Seed. In the seed the greatest change is in the inward growth of the endosperm, and its extension backward beyond the secondary chalaza. The centripetal growth proceeds at first at the chalaza (f. 10) filling the cul-de-sac; the inwardly moving walls then meet at the upper end, the fusion progressing toward the opposite (micropylar) pole of the embryo sac. All nutritive changes in the endosperm progress, similarly, from the chalaza toward the micropylar pole. When the space is entirely filled, and before secondary changes set in, the endosperm cells are very thin walled, with the nucleus suspended in the middle of the cell by numerous radiating protoplasmic threads. They are isodiametric, but their radial measurements begin to increase rapidly, concurrently with the later growth of the seed. The growth of the integuments is very rapid, in view of the rapid increase in volume of the seed, and their definitive characters are still absent.

The embryo at this time is spherical in form, 60-75 microns in diameter; at the time when the cavity is just obliterated, the seed measures 2.5 mm. in diameter in the transverse plane in which the embryo lies. The increase from this time, to a transverse diameter of 8.5 mm., in the ripe seed; and from a length of 8-9 mm. to 28-30 mm., is the period of maturation, anatomically speaking, during which the greatest changes are to be found in the endosperm and embryo. There is usually still more torsion, which brings the embryo into the ultimate position, about midway the seed. The position is, however, variable and may occasionally be quite abnormal. From the circular embryonic area, visible on the outside of the seed, a slender line may be traced downward. This is the evidence of the torsion, and is the anatomical indication of the micropyle.

Stage IV. Close of the Third Period.

Carpel (f. 32, 33). The definitive epidermis, 15 microns deep, is heavily cutinized, the cuticle 4 microns thick. The cells are nearly isodiametric and straight walled. There are occasional stomata, each supported by four or five accessory cells. Beneath the epidermis is a thin layer of parenchyma 45 microns thick, of four to five layers of tannin cells, compressed radially. Within this layer again is the zone of stone cells, occupying a very irregular thickness (60-120 microns) according to the position of the individual cells. The longer ones are placed radially. It will be convenient to regard these tissues as constituting the exocarp. The mesocarp is composed of very thin-walled parenchyma, with here and there a raphide, or tannin idioplast, and penetrated longitudinally by vascular strands. The midrib of the carpel is marked by a median vascular strand, and the suture by a dipping in of the layer of stone cells (f. 11). The inner zone of the mesocarp is composed of more or less crushed and disorganized parenchyma, which, in the ripe fruit is conspicuous as loose, shining, fibrous masses (the "rag"). These are only loosely attached to the endocarp, and constitute an irregular, poorly delimited zone which

must properly be regarded as part of the mesocarp. The endocarp is a definite, rather tough membrane (f. 34), composed of the inner epidermis and more or less underlying tissue of compact cells. This membrane adheres somewhat firmly to the seed but splits readily longitudinally on removal.

Along the side of the placental ridge there are two parallel strands of endocarp epidermis which are especially obvious in chromic acid and ethyl nitrite material. They appear to be differentiated on account of their anatomical relation to the underlying placental tissue which is rich in tannin. It is possible, therefore, that they represent transfusion areas, where the tannin passes from the carpel into the ovule.

Seed. The outer integument (f. 31). The epidermis is completely sclerosed, having pitted walls. The cells are, on the whole, elongated parallel to the axis of the seed, but at the same time they show *en face,* a very great deal of torsion and irregularity. There is an irregular hypoderm of more or less similarly sclerosed cells, beneath which are thin-walled tannin cells. It may be an expression of physiological correlation that tannin is not to be found in the sclerosed cells. The inner integument is somewhat difficult to delimit sharply; it consists of at least two layers of cells, the outer and inner epidermis, with usually one or two additional layers of compressed cells between. The entire integumental covering measures about 30 microns in thickness, but thickens toward the raphe and above the embryo.

Directly impinging upon this, and within it, is the thick-walled endosperm. The outermost cells are very frequently isodiametric, are thinner walled than those within, and have few pores or none. The longer ones have pores at their inner ends, or on the sides when unusually long. All the cells radiate toward the morphological middle of the endosperm, which is marked by a mass of isodiametric, roughly spherical cells.

The embryo is short and cylindrical in form, but is frequently distorted longitudinally. It is placed at right angles to its original position. The end toward the axis of the seed

is the haustorial cotyledon, with which numerous students, beginning with Malpighi and Sachs, have familiarized us, and may be somewhat enlarged, when seen in a longitudinal section of the seed. In transverse section, this end of the embryo is usually smaller than the radicular end. The whole embryo measures about 2 mm. in length, of which one-fifth is the hypocotyl. The radicle proper is scarcely .3 mm. long.

The form of the seed, as seen in transverse section, varies with the race. In Deglet Noor, the outline is nearly circular, but may be disturbed by a low, lateral ridge. This is very marked in Rhars, so that the outline is lobular.

OCCURRENCE AND DISTRIBUTION OF STARCH, TANNIN AND OIL.

STARCH.

Starch is to be observed, in the form of transitory grains, only during a relatively brief period of the earlier developmental stages. Its appearance at all seems to be connected with the slower rate of growth prior and immediately succeeding pollination. During the later period of rapid development of the fruit, none appears at any time.

Observations.

Nondescript. May 16, unpollinated. Starch in the upper zone of pedicel, just beneath the receptacle, in large grains. Also in the inner (ventral) part of the parenchyma of the outer bracts, and in all the parenchyma of the inner bracts toward their bases, and in the ventral moiety in the basal half. In the receptacle below the insertion of the pistils, but much less in a zone above the pedicel. Pistil: Rather abundant in the basal part, becoming reduced in quantity in the upper half, where it is of very minute grains. Similarly minute grains in the ovule, in the funicle, outer and especially in the inner integument. The amount of starch is greater near the nucellus, becoming reduced to none toward the periphery. There is no starch in the tannin idioplasts in the carpel or elsewhere.

The same material one day after pollination showed the same distribution of starch.

Tronja. May 16. One day after pollination gave similar results qualitatively but much less marked.

Deglet Noor. Pollinated, April 6; fixed, May 16. Starch in a few scattered grains in the pedicel in the parenchyma adjacent to the bundles, in the parenchyma at the base of the carpel, and in the inner integument between the funicle and micropyle.

Nondescript. Pollinated, Apr. 11; fixed, May 28. Starch in the ovule restricted to the basal part of the funicle and to inner zone of outer integument.

Deglet Noor. Pollinated, April 6; fixed, May 16. Starch restricted to the mass of tissue between the funicle and micropyle and to a small volume of tissue along the funicle nearby. More in the base of the carpel itself.

Up to this time the growth of the ovule has involved chiefly the chalazal half, from which concurrently the starch has disappeared, and in which it never reappears.

It is thus seen that this form of carbohydrates plays only a brief rôle during the embryological period. The deposition of starch appears to be inconsistent with a very rapid development such as characterizes the date fruits after the seventh week following pollination.

The course of the disappearance of starch seems to be connected, in part, with the general growth of the ovule, as well as with its removal into the embryo-sac.[7] The latter undoubtedly accounts for a part of it, but that which remains in the outer integument is probably consumed during the earlier phases of growth in this structure.

Embryo. Starch appears in the embryo at first at a comparatively last stage of development. At 17 weeks (f. 17) a very small amount is seen in the root cap and in the apex of the cotyledon. During the embryological history starch is unimportant quantitatively; during germination as described by Sachs (1862) its importance increases.

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[7] Ikeda, 1902.

TANNIN.

One who now-a-days makes use of the blanket term tannin throws himself open to criticism. Nevertheless, it is not always possible to avoid it, especially when blazing the way into material necessitating the use of histological methods. A distinction has been drawn between "plastic" and "aplastic" tannins, and objection has been raised[8] to the use of these terms. They explain themselves, but it is obvious that we can use them only to describe tannins in a particular structure when they have been shown, in the end, to remain definitively, or to disappear during metabolism. "Aplastic" may, despite that objection, be used merely as a matter of temporary convenience to designate that tannin which appears in a particular situation to remain permanently thereafter. Conversely, plastic tannin is that which appears only to disappear. Such tannin is analogous, in appearance at least, to transitory starch, and the evidence to be offered indicates that the comparison is justified by more than appearance alone. If the attempt is not made in what follows, to distinguish chemically the tannins with which I have concerned myself, this is due to the conditions and, not less, to the point of view.

The history of the study of tannins is extensive, and the materials which have been studied profuse. Happily, Dekker (1906), in a most painstaking way, has brought the available data into the compass of a small but rich work, and for this the thanks of the botanical fraternity are due him. A perusal of the historical survey in Dekker's paper shows that there are two general views held as to the physiological rôle of tannin. These are, obviously, that tannin is a waste product, and, opposed to this, that it is related to glucose and is of use in forming more complex carbohydrates than itself. Doubtless both are true, but, doubtless also, the same substances are not meant in all cases. Thus, Sachs (1862) regarded the tannin in the germinating date seed-

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[8] Pfeffer, Physiology of Plants. p. 493.

ling as an excrete, and the evidence supports his view. Contrariwise, Servettaz (1909) has observed that tannin occurs in certain situations in the young, rapidly developing ovule (in the Eleagnaceae) and argues, with equal right, that here this substance is concerned with nutrition. In view of these observations, it would seem the rational procedure to regard the tannin which accumulates, and remains permanently, in certain cells of the germinating date as different chemically from that in the young ovule of the Eleagnaceae. The chemist may tell us later that, indeed, the former is tannin, and the latter not. These two instances only are cited, because as we shall presently see, they are pertinent to the matter in hand. For the present purpose, we must be content to call those substances tannins which afford us the visible reactions which are usually resorted to, and rely upon observation to tell us whether a particular tannin is an excrete or a nutrient.

Aplastic tannin is known to occur in the date. Thornber[9] studied a series of date fruits from the "size of a pea until full ripeness" and came to the conclusion that there is "no general distribution of tannin" but that it is "strongly segregated in a layer of very large cells near the surface of the fruit and, especially in the younger stages, near the seed." Avoiding hypercriticism as to the meaning of "near the seed" these facts have been further mentioned and illustrated by Vinson (1910) for the purpose of demonstrating the value of ethyl nitrite vapor in the study of tannin in plant tissues. Vinson believes that "a green date may also be easily divided into astringent and non-astringent portions with a pocket knife" and has said to me personally that, before ripening, the portion (mesocarp) lying beneath the layer of idioplasts (f. 32) is not astringent, but that the contents of the idioplasts may be expelled by gentle crushing and appear mucilaginous. The inference is at hand that the tannin is confined wholly to the idioplasts, but, for reasons which will be given beyond, I think this is not quite

[9] 1906. Not over his personal signature.

true. Howard (1906) on the other hand concluded from his study of the ripening persimmon that before ripening the tannin is distributed in the cells of the "loose parenchyma where the tannin cells are located" as well as in the idioplasts. During ripening the tannin, according to Howard, "becomes condensed in certain specialized cells" (Bigelow, Gore and Howard, 1906, p. 702), and there becomes insoluble. The observed facts in the date (Lloyd, 1907),[10] as we shall see, do not accord with this view, though in the date as in the persimmon, either during natural or artificial (Vinson, 1909) ripening, the tannin becomes insoluble,[11] and hence the lack of astringency in the fully ripe fruit, in these as in other cases, *e. g.*, the sapodilla, according to Geerlig (1909). What is true of the specialized tannin cells, in this regard, is true also of the remaining tannin cells, those namely in the inner mesocarp and endocarp, and, also, in the integuments, since they are alike tasteless in the ripe fruit.

The only remaining observations on the date which touch on the following account are the following: Pond (1907) reported finding tannin in the membranes (endocarp) about the seed, and cautioned students of digestion in the germinating date against the introduction of tannin from the integuments into the fluids to be tested for reducing sugars. Sachs (1862) discovered tannin diffused through the tissues of the resting embryo, and described the final accumulation of such material in certain parenchyma cells of the seedling.

Reference has been made to Servattaz' observations on the Eleagnaceae. The tannin, *"substances tannoides,"* he says, occurs in the ovule, in the elongated cells of the nucellus at the base of the embryo-sac and connecting this with the vascular tissue of the raphe. It occurs also in the mid-layer of cells in the external integument. Continuing, he says, "Dans l'ovule, ces substances ne prennant pas naissance à la suite de la destruction de l'amidon, car il ne s'y forme jamais de réserves amylacées. Le glucose abonde dan toutes

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[10] Not over his signature.

[11] Mylius (see Dekker, 1906), appears to have made observations to the same effect, but I have not seen his paper.

les parties de l'ovule . . ." (p. 353). Servettaz, further, inclines to the belief that the tannin may be employed in the nutrition of the embryo-sac, and advances the interesting view that antipodal cells play an active rôle in the process: ". . . les tannins amenés à la base du nucelle seraient transformés en glucose par la 'cellule antipode' qui occupe l'extrémité du sac embryonnaire, car cet organe ne renferme pas de tannins . . ." (p. 411). He concludes that, though tannins occur in such situations that they must be considered a waste, he also takes the position that they are in other situations useful, and cites the finding of Gerber (1897), who has shown that tannins give rise to glucose during the maturation of some fruits.

In what follows it is hoped to show that (1) in the date tannin occurs in special cells in such a state that we are precluded from supposing that it serves any but secondary functions, such as protection, as shown by Vinson (1909). This tannin is aplastic. (2) That there is no condensation of tannin just prior to ripening, meaning by this a movement of tannin from the surrounding parenchyma to the special cells or idioplasts. (3) that tannin (plastic) occurs and disappears at such times and in such situations as to warrant the conclusion that it is a nutrient, and in this is analogous to starch, reserve cellulose, oil, etc.

Carpel. Hypodermal tanniferous zone (f. 33). The parenchyma cells lying between the epidermis and the zone of stone cells are more or less flattened radially, and take up, with the epidermis, a thickness of about 60 microns, of which the epidermis accounts for 15. In the *Rhars,* the majority of these cells, usually excluding the epidermis, contain tannin, and they constitute a zone one to three cells in thickness, immediately beneath the epidermis. Occasional cells among them are sclerosed and may also contain tannin. In *Deglet Noor* there is a similar zone, but usually of only one cell in thickness. The hypodermis of commercial dates displays the same tanniferous layer, the cells containing tannin "vacuoles," or masses lying in the vacuoles. In material fixed with a chromic acid mixture, the tannin does not ap-

pear in the vacuole in all cases, but in or against the protoplasmic lining.

In addition to the tannin in the more conspicuous tanniferous cells lying immediately beneath the epidermis, a small amount of tannin occurs in all the parenchyma to a depth of 180 to 200 microns inside the zone of stereids (f. 33). This tannin is always in the form of minute droplets scarcely ever more than 6 microns in diameters and for the most part smaller than this. The cells themselves show no peculiarities, and in no way are differentiated from the remaining parenchyma within, except in containing these droplets of tannin. Thinking, upon discovery, that they might be oil, I tested them with alkanet, with negative results. Upon allowing the section to lie over night in very weak methyl blue, they became very deeply stained. Their color reactions are identical with the more abundant tannin of the hypodermal or idioplast layers, but they are not conspicuous on account of their small size. Their occurrence does not extend as far as the idioplast layer, nor do any of the parenchyma cells near to this layer show the presence of tannin droplets.

Origin—Indications of this tannin zone are to be seen after pollination. One week thereafter, distinctly differentiated hypodermal columnar or cubical tanniferous cells may be seen, though as yet the zone is not continuous. At the time of pollination, these cells are not yet differentiated, but the epidermis shows a diffused tannin reaction. With the dying back of the stigma and very short style, the tannin cells appear rapidly. These cells, constituting the hypodermal tannin zone, are sharply differentiated once for all, and differ from the idioplasts only in their lack of special structural character. They are distinguishable only by their contents.

Sub-hypodermal tanniferous zone (f. 32). This zone is composed of large idioplasts (giant cells[12]) and is of varying thickness (1 mm. more or less), lying about 1 mm. below the surface of the carpel.[13] The layer is four to six cells

[12] So called by Swingle, according to Howard *l. c.*

[13] The character of the zone varies considerably with the race. A few of the difference are illustrated by Vinson, 1910.

deep, roughly speaking, in Rhars and Deglet Noor, the number of the cells depending on the size of the elements as well as upon the number which may be counted in radial direction. Tannin similar to that in these cells occurs also in the integuments of the seed, and in the mass of tissue, partly of integumental, of chalazal and of funicular origin, which fills the deep prominent groove characteristic of the date seed.

Origin of the layer of tannin idioplasts. This layer makes its appearance at a very early stage of development, some time before pollination. For the present purpose it is sufficient to sustain my contention to show its condition prior to pollination and its development thereafter. Both fresh and preserved materials including nitrous ether preparations, were studied.

At this time the layer in question is continuous from the base of the carpel, upwards, overarching the ovule. At the base the component elements are nearly or quite spherical, and about 15 microns in diameter. Toward the base of the style, where the elements are the largest, they reach a size of 45-60 microns. The arch is interrupted at the apex by the stylar canal and its immediately surrounding tissues, and here also the thickness of the layer of four to six cells is the greatest. Toward the base of the carpel, it dwindles to the width of a single cell. The layer is homologous with the similar tissues in the bracts surrounding the three pistils, and appears the same physiologically. Precisely the same condition is found in *Chamaerops humilis*. Tracing them through the succeeding stages of development, there is no change in the constituent elements, except an increase in the amount of their tannin content. Other cells are added to the tissue, being intercalated between those already present, or in a radial direction.

The contents of the cells either lie in a layer about the wall, or partially fill the lumen, and are highly vacuolated,[14] this, as we shall presently see, in contrast to the same cells later on. In material which has not been prepared with a

[14] Due perhaps to the liberation of gas during the reaction.

view to the detection of tannin, they offer no points of striking contrast so that they may be distinguished from their neighboring parenchyma only by their size, and in this respect resemble the primordia of the stereids, which lie, also in a layer, between the tannin cells and the epidermis. Both the former and the stereid primordia are parenchyma cells which become secondarily specialized, and both have this in common also, that once their peculiar content is laid down, it remains unchanged: namely, in the tannin cells, tannin of the fixed variety, and in the stereids, cellulose and lignin.

What appears however in this condition as a simple peripheral layer of protoplasm, must be in a living condition, as shown by the proper reagents, protoplasm imprisoning a solution of tannin. Alcohol acetic material, even after prolonged treatment, does not seem to be depleted of the tannin, for upon the addition of methyl blue, a fine, flocculent precipitate is discoverable, occupying the whole of the interior vacuole. Material treated with copper acetate displayed a variety of curious forms of solid tannin-copper compound, and these are, I believe, to be regarded as artefact. Thus, sometimes a coarser or finer network of tannin forms a sponge-like mass, filling the interior; or into this may extend, from a solid, continuous lining, arms of the same; or the whole interior may be filled with a vacuolated mass; or finally it may appear as a complete, homogeneous lump filling the whole of the cell as seen in ethyl nitrite material, as well as in that prepared by other reagents. In these conditions, one may hardly discover the protoplasm which is responsible for the secretion from this, and for this purpose copper acetate, with subsequent treatment with other metal salts for color effect, is really inadequate. Much better is methyl blue in this regard.

Not however to do the copper acetate method too great injustice, I may add that sometimes a quite fine precipitate is to be seen, though the protoplasm is still not to be successfully differentiated to the eye.

To return to the anatomy of the tannin idioplast layer.

While generally continuous, and forming a zone of fairly uniform thickness there is, along the line of carpellary fusion, a scattering of the cells. Single cells, or groups of these occur in all the tissue between the inner limit of the carpel, and the outer limit of the zone in question (f. 11). In the condition before pollination, this dipping inward of the tannin tissue is not obvious, for the reason that there is little thickness of the carpel here. But with this secondary thickening following pollination, the number of tannin cells increases within the region of carpellary fusion, as within the idioplast zone proper.

At maturity, the number of tannin idioplasts has increased enormously, while, also, the size of the elements is also much greater. The largest are upwards of .2 mm. in radial measurement, and over .1 mm. in diameter, though these measurements must be taken as indicative of the generally large size, rather than a specific statement of size, which varies with the race. I measured the tannin cells in a fruit of Deglet Noor.

Little further needs to be said by way of description of these cells. One may remark, however, that an examination of a full series of material suitably preserved, extending over the whole period of maturation from April to October, taken at intervals of two weeks, shows no evidence of the secretion of tannin within the parenchyma adjacent to the idioplasts indicating that these latter act merely as receivers of tannin or tannin-like material. If such is the case the evidence would be discoverable since it has been obtained elsewhere in the fruit and seed.

Intercellular Tannin.[15]—A peculiar condition is not infrequently to be observed, namely, the injection, as it would appear to be, of the intercellular spaces of the idioplasts and adjacent tissues with tannin.[16] I have been unable to fix upon the explanation of this though there is some evidence

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[15] According to Winckler, extracellular tannin pockets occur, but I have been unable to see this paper.

[16] Howard (1906, p. 570); speaks of this condition, in the persimmon; I have recently confirmed his observation.

that it is due to bruising of the fruit. The masses of tissue showing this condition are irregular, but always associated with the idioplast layer. Aside from this, they are not at all constant in position. Wherever the surface of the fruit has been slightly damaged by pressure, as shown by brownish coloration, one may generally find more extensive injection of the intercellular spaces beneath than elsewhere. Where actual wounding and tearing of the tissues have intervened, there may be observed a still more pronounced appearance of the tannin in question. Whether however the injection of the intercellular spaces is due to an excretion or to rupture of the tannin-containing cells there is no evidence to decide. Vinson has observed that mature dates rupture on being placed in certain fluids, and this occurred in some of my own material, as well as some shrinkage at times. The unequal pressures that are set up would probably be sufficient to bring about the results described.

Similar injection of the intercellular spaces frequently occurs along the sutural tract, in contact with the idioplasts occurring there (f. 11).

Mesocarp; endocarp. The line of delimitation between the mesocarp and endocarp is not definite. It will, however, make a clear enough anatomical distinction to regard the toughish membrane which frequently adheres somewhat to the seed as the endocarp. This may vary in structure in being of from one to several cell-layers in thickness. The cells are somewhat elongated with square, oblique or irregular ends, occasionally sclerosed, and not infrequently, as Pond (1907) discovered by chemical methods, contain tannin which is similar to that found in the idioplasts.

The mesocarp embraces two zonal regions, the outer and inner. The outer mesocarp shows no peculiar anatomical changes in the later period of development. That sector of it which includes the tissues along the line of fusion contains a good many scattered idioplasts, similar in all respects to those of the periclinal zone, except that they are more or less elongated. The closer they lie to the seed, the greater their length; so great, indeed, that they are only

with difficulty recognizable as of similar origin to the isodiametric idioplasts themselves.

The inner mesocarp may, for our present purpose, be described as a mixture of collapsed, chiefly elongated, thin-walled elements penetrated by a few displaced and distorted vascular strands. Here and there, isolated or in small groups, are very long cells rich in tannin. Sclerosed cells of similar form, having dimensions upward of 10 mm. in length and .2 mm. in diameter, their walls penetrated by simple oval or circular pores, and vaguely obliquely striated, are also to be found, more especially opposite each lateral angle of the seed, where a group readily visible to the naked eye, may occur. In transverse section, these stereids are circular or oval and sometimes contain tannin (f. 34).

Additional tannin is occasionally observable also throughout the mesocarp elements and bundles, in both hadrome and leptome. Frequently vessels filled with tannin have been seen. I am unable, however, to make a sufficiently detailed account of this phase of the matter to warrant any definite conclusions. This would require a more particular study of the vascular tissues than I have been able to give them.

There is some evidence that plastic tannin occurs in small amounts in the parenchyma of the mesocarp in certain regions. Copper acetate and ethyl nitrite material frequently displays a copper-red or orange coloration respectively, which blackens with iron salts. As maturity is approached, this behavior is not to be observed. Fresh material was not studied, and the facts just stated may be misleading, but it would seem to be the case that plastic tannin, similar to that to be described in the endosperm, occurs in small amounts in the mesocarp during development. The reaction upon which this conclusion is based is more marked in the sutural sector than elsewhere, and in the inner than outer mesocarp. In the latter, however, the amount, as previously stated, is exceedingly small at any time. For some time previous to ripening, as attested by Thornber, Vinson and myself, no tannin occurs in the major portion of the mesocarp. There is, therefore no evidence in this instance that tannin gives

rise to any of the abundant sugar, one of the alternative views advanced (Bigelow, Gore, and Howard, 1906).

Ovule. Stage I. At the time of pollination there is tannin distributed throughout the whole of the ovule, as shown by a diffuse reaction. There is, however, a much greater quantity in the embryo-sac, inner integument, chalaza, raphe and pedicel (f. 2). The endocarpal pollen-tube guiding tissue shows a similar amount of tannin.

Regarding, for a moment, the anatomical facts recited above, it is difficult to avoid the conclusion that there is special nutritive activity connected with tannin both at the antipodal end of the embryo-sac and in the tapetum. In the Eleagnaceae (Servettaz, *l. c.*), while tannoid substances occur in the nucellar cells beneath the antipodal cells, and do not occur in the embryo-sac, it is argued that the antipodel cells play an active rôle. The conception of the physiological importance of these cells, in spite of their frequently small size and lack of histological peculiarities, is one to which we have become accustomed through the work of Westermaier, Goldfluss, Lloyd, and others,[17] but it still remains to determine in detail what their method of operation may be. Servettaz' suggestion should prove fruitful.

In the date, although the antipodal cells are small, their anatomical relations are peculiar. In the definitive embryo-sac, they are placed at the end of a slender pit, surrounded by elongated cells with thickened walls (f. 4). The thickening appears to be due to swelling, and this must, I think, be of significance since it is only the walls forming the antipodal pit which are swollen (f. 1, 3). Whether this is the same sort of thing observed by Servettaz (*l. c.*, p. 354) or not, I cannot say. He appears to regard the thickening and gelatinization of the antipodal cell walls observed by him as an accompaniment of disintegration. In the case before us, the swelling of the cell walls is not due to digestion, since their persistence (f. 5) shows the contrary. They appear, however, to be full of tannin, as they show a definite reaction

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[17] Coulter and Chamberlain, Morphology of Angiosperms.

at once on the addition of reagents.[18] A careful study of the development and nutrition of the embryo-sac previous to pollination will, I believe, show that they behave in some such way as suspected by Servettaz in, *e. g.*, *Hippophae rhamnoides.* Unfortunately my material has not been suitable for this.

I have been able, however, to follow the behavior of the antipodal cells after pollination. It appears that disintegration proceeds from the basal cell, operating successively on the adjacent and the innermost (f. 4, 4a). The disintegration is preceded by the reduction of the nucleolus, and the enlargement of those of the adjoining cells. The order of disintegration is the reverse of that followed in *Hippophae* and other Eleagnaceæ (Servettaz, *l. c.* p. 354). Whatever the explanation of the difference, their position at the end of the elongated cells which represent the end of the vascular supply to the embryo-sac, as in *Hippophae,* the presence and evident nutritive importance of tannin, the peculiar thickening of the contiguous cell walls, and their ultimate behavior, all offer positive evidence of their physiological importance.

It would similarly appear that the tapetum[19] is also important. At the early stage of development both tannin and starch occur in the inner integument and tapetum. This tissue does not break down—is not digested by the embryo-sac. The character of the tapetal cells indicates that its nutrients are transferred to the embryo-sac, following the decomposition of the thin nucellus.

Just before, or, at any rate, immediately following pollination, the endosperm begins a rapid chalazal growth, previous to the division of the secondary nucleus. In doing so, it digests the tissue at the base of the embryo-sac quite irregularly, and forms several cul-de-sacs which extend backward beyond the antipodal pit. This is, for some time, left projecting into the utriculum of the embryo-sac (f. 5).

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[18] This is not the secondary staining of the wall spoken of by Strasburger in his Practical Botany.

[19] Goldfluss, 1899.

Finally the tissue beneath it is undermined, and so a single antipodal cul-de-sac is formed, which continues its growth upward and toward the raphe. During this time the tissue about the cul-de-sac shows a reducing action on Fehling's solution. The cell walls are swollen, and stain deeply with Bismarck brown. Thus we may infer that active digestive processes are in progress; but we are not, of course, able to say whether the reaction is one to tannin alone, or to a reducing sugar, or both. The cul-de-sac is therefore a haustorium, for a prolonged period constantly encroaching upon the surrounding tissues. Its size increases as the seed enlarges till its dimensions are sufficient to make it a prominent feature of the mature seed (figs. 5, 6, 7, 10, 13, 12). It is important to notice in this connection that, resulting from the stimulus of the growing cul-de-sac, the surrounding chalazal tissue increasing concomitantly by cell division, its cells become radially disposed. These always contain tannin, the least densely filled cells being those next to the endosperm. A part, at any rate, of the food material supplied to the endosperm is a tannoid substance and this passes over into the endosperm chiefly at the cul-de-sac. As we shall see, a tannin is also present in the endosperm during some part of its development. That the chalazal cul-de-sac is the active center for the distribution of nutrients is indicated also by the circumstance that it is the center from which progress the physiological as well as anatomical changes which overtake the developing endosperm; and by the further fact that the remaining tissues contingent on the embryo-sac grow, *pari passu,* with the endosperm.

Why the endosperm digests the tissue touching it at one point and not at another is an interesting and important question, which has received little attention. The same question applies to the embryo-sac, during its development in many instances; and similarly to the developing embryo. I have elsewhere[20] shown that the embryo-sac may develop in a quite abnormal manner in this regard and behave much as a pollen tube, but in a more aimless fashion, so to speak.

[20] Lloyd, 1902.

The facts observable with the microscope seem to force the conclusion that the mechanical conditions determine the behavior. Thus, in certain Rubiaceae, the embryo-sac extends forward along the micropyle, during the development of the single thick integument. If, as may happen, instead of remaining within normal bounds, it continues to grow until it extends beyond the exostome, it then pushes its way in any direction—the easiest mechanically. But if, instead of moving forwards, it develops backwards into the chalazal tissue, its path is indirect, and appears to be wholly indeterminate. In the date, however, the backward growth of the endosperm is direct and determinate. To be sure, it is pointed in the right direction to begin with. Though it digests the nucellus—a very thin layer—and to this extent moves forward, it is resisted by the integumental tapetum. So far the same phenomenon is seen in other plants (*Elaeagnus,* Servettaz, *l. c.* Cucurbitaceae, Kirkwood, 1904) but to a more marked degree. This resistance may, probably, be explained by a chemical response, or by a resistant layer, say a cuticle, as observed in *Tricyrtis* by Ikeda (*l. c.*). But how shall we explain the direction of the antipodal haustorium, unless we assume, as a working hypothesis, that the response is chemotactic and that, therefore, many facts about the embryo-sac are to be explained in the same way that we explain the behavior of the pollen tube! To assume a local enzymatic activity would be, I think, to assume too much, though this also is not impossible.

Later stages (the young seed). During the whole period of development until maturity the raphe and integuments contain iron-blue tannin. For some time—no exact period can be determined— the reaction in the integuments is diffuse, as it is in the bulk of the raphe, in which, however, are groups of cells showing a superior content. The appearance of these cells suggests that the tannin in them is aplastic, while their position, which is removed some distance from the endosperm, also indicates this to be the case. Similarly, as stage III is approached, there is evidence below the level of the embryo, that the tannin is accumulating in permanent

form in some cells. At this time (f. 14) the endosperm cells in contact with the raphe and the integument contain more tannin than the inner ones, and it would seem that the integuments serve in some measure to distribute the tannin. The markedly greater activity in this regard of the chalazal tissue has already been noted.

As maturity is approached, many of the cells of the outer integument lose their tannin. These are chiefly the epidermis and an irregular zone of underlying cells, in all of which not the least trace of tannin is to be found (f. 31). The cells of the inner integument are all tanniferous. What becomes of the tannin in the cells which definitively do not contain it is not easy to say. There is no evidence that it is removed to adjoining cells; on the other hand, there is none that it is transformed, beyond the circumstance that there is a considerable sclerosis of the epidermal cells. It has been noted that of cells of similar origin, apparently, in the endocarp, some become sclerosed and do not contain tannin (or occasionally, very little), while others remain thin walled, and are tanniferous. This lends some probability to the view that the tannin is used in physiological changes in the individual cell. If, however, the removal of tannin from the epidermis and underlying cells is to be explained by lateral movement into those cells which are finally tanniferous, this happens long before the period of ripening, and is not the rapid segregation described by Howard (*l. c.*) in the pericarp of the persimmon.

After ripening, the cells of the integument and raphe, with the exception noted, are heavily loaded with insoluble aplastic tannin. To this the deep brown color of the seed is due.

To sum up briefly, it appears that the tissues of the ovule, setting aside the endosperm for the present, are, during the period of growth, supplied with plastic tannin which is passed over to the developing endosperm. During the same period, tannin cells containing aplastic tannin appear, few at first in the raphe, but at length throughout both raphe and integuments. During the latter part of the interval be-

tween stages III and IV, tannin disappears from the outer part of the outer integument. All the tannin finally becomes insoluble; it is found to be so in the ripe fruit.

There is evidence that during development tannin moves from the integuments and raphe into the endosperm, as well as from the chalaza, where the activity is especially evident (f. 27a).

Endosperm. Stage I. A strong tannin reaction is seen in the embryo-sac. Just at the time of pollination the endosperm nucleus and its protoplasm become blackened by iron following copper. The same evidence is had by the use of ethyl nitrite, which gives a brown reaction. Alcohol material gives a less pronounced, but readily observable reaction. For a period of eight weeks, or somewhat longer, the endosperm retains a syncytial character, and remains as a thin protoplasmic lining. All the material collected during this period (Rhars and Deglet Noor— in alternate weeks) shows that during the whole of this time, the endosperm is always replete with tannin. Copper acetate and ethyl nitrite material invariably show also a thick precipitation membrane lining the endosperm utriculum, from which it must be argued that the fluid within was rich in soluble tannin. It is also to be inferred from the facts of distribution, that the tannin is diffusible, and this is supported by a study of the endosperm in a later stage.

Stage II. From this time on the endosperm develops centripetally (f. 10), but this more rapidly in the chalazal cul-de-sac than at the micropylar end. During the periods of growth between stages I and III, there is a general distortion, producing a displacement of the embryo, so that this comes to lie definitely in the familiar midway position. (f. 12). The filling of the utriculum occupies about four weeks, in which time the seed attains a length of 9 mm. with a diameter of 3 mm. (Deglet Noor). The lack of any pronounced tannin reaction (more marked in the peripheral cells than elsewhere) at this time, indicates that there has been a substitution of one material for another. As a matter of fact for the first time there is to be noted a general

distribution of oil in small droplets throughout the endosperm, but whether there is any causal relation to be traced is not to be decided off-hand.

Stage III. Very soon the thickening of the cell walls, to form reserve cellulose, begins, (f. 26 a). Concomitantly, there is *a reappearance of tannin,* so that, in material two weeks older, (3 months old) there is tannin throughout[21] the endosperm in large amount (f. 27). The amount is however much greater in the central portion, namely in those cells, strictly speaking, which are undergoing secondary thickening of the cell walls, looking toward the definitive condition of the seed. The reaction at this time is very pronounced, and the tannin-bearing portion of the endosperm forms the most conspicuous feature of the seed macroscopically (f. 27).

As I had some doubt of the facts here presented, another lot of material was obtained for me by Professor R. H. Forbes, and treated by Dr. A. E. Vinson with ethyl nitrite. The material, Deglet Noor, included material pollinated March 9, and fixed June 9, 1910. Upon examining this, the whole of the endosperm and the embryo were perfectly white, a quite unexpected result. The area (shown by stippling in f. 28) which should have been deeply stained, was a more opaque white than the surrounding zone. But upon the addition of ferric chlorid, a prompt reaction followed, in quite the expected manner. Upon prolonged treatment with very dilute ferric chloride, the central part of the endosperm was stained dense blue-black, with the outer zone obviously less so. Re-exposure to vapor of nitrous ether produced the reaction also, from which we may infer that the period of original exposure was insufficiently prolonged.

During another two weeks the seed grows rapidly, attaining a transverse diameter of 7 mm. (f. 29), and the tannin tissue has extended toward the periphery, but is less pronounced in its response to reagents. After this, the density of the reaction is quickly reduced, but extends quite to the

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[21] See also p. 134.

periphery. I have found a relatively small amount of tannin present even as late as October 8, in Deglet Noor material pollinated on March 28. Frequently the disappearance of the tannin is even in rate throughout the whole mass of the endosperm, but one more often observes that it is at different rates in different parts. In some instances (f. 28, 30), and these are particularly instructive, the tannin remains longer in certain sectors, having their vertices at the line of fusion marking the obliteration of the endosperm utriculum, than in others. The wedge-shaped areas (f. 28, 30) thus made conspicuous, reach quite to the outermost layer of the endosperm, and their superior tannin content is quite evident to the unaided eye. In other cases, similar but irregular areas occur, sometimes centrally placed and showing a radial disposition, or it may be less definite relations. On the whole, the tannin is retained rather longer in the general region of the raphe than elsewhere, thus suggesting a movement of the material concerned from this part of the ovule into the endosperm. The last evidences of tannin are to be seen in the outermost, peripheral endosperm cells, which are the last to take on their definitive character. These are at this time passing from an obliquely distorted condition, due to shearing pressures exerted by the endosperm on the one side and by the integuments on the other. With further growth they finally attain a radial disposition, and the tannin content entirely disappears, save in isolated positions. The mature endosperm shows very little tannin, and this is confined to irregular sulci, caused by uneven rates of growth and the consequent tangential crushing of peripheral portions of endosperm which are unable to thicken their walls, and to adjust themselves finally to the surrounding tissues. The minute quantity of tannin found by Pond (1906, p. 74) is probably to be explained by this circumstance, though a partial explanation may be had in the incomplete eradication of tannin elsewhere.

From the circumstance that there are no special tannin cells in the endosperm at maturity, we must conclude either that it disappears as the results of chemical change or that

it migrates into the surrounding integuments. The facts described above do not consist, I believe, with the latter alternative, but are quite applicable to the former.

The appearance of tannin simultaneously with the beginning of secondary thickening of the endosperm cell walls has a good deal about it to warrant the belief that the tannin plays some rôle during the formation of reserve cellulose. The reaction is not in the cell lumen, but definitely in the cell wall, and, in the more deeply reacting cells, in the primary membranes. This appears not to result from displacement of tannin[22] as the reaction appears at first in the cell wall. In copper acetate material, in which we may imagine displacement of tannin from the lumen into the wall to have occurred, the tannin reactions in other tissues (e. g. the raphe) are in the lumina. Ethyl nitrite material gave identical results in the clearest and most convincing fashion. The endosperm, as stated elsewhere, did not show coloration, but, upon the application of iron acetate, gave a prompt, iron-blue reaction in the cell-walls, but not in the lumina. In the integuments and raphe, a similar reaction was given in the lumina of the tannin cells.

This parallelism between the distribution of tannin and the thickening of the cell walls, and the ultimate disappearance of the tannin when the endosperm is fully developed, indicate an analogy between the tannin in question and other nutrients in transitory form. For this view speaks the fact that the tannin in the endosperm is throughout diffusible, since, in contrast to the tannin in specialized cells, it is found permeating the cell walls. This conclusion is justified from another point of view. Strecker, according to J. Reynolds Green[23] has shown that a "tannin" is a glucoside, yielding gallic acid and glucose by hydrolysis. The fermentation of infusions of certain galls, yielding, with the disappearance of tannin, gallic and ellagic acids, (also pointed

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[22] See above, p. 132.

[23] The Soluble Ferments and Fermentation, p. 160. See also Dekker, 1906.

out in Green's work) taken together with the hydrolysis just mentioned, indicate that the comparison of the plastic tannin in the date endosperm with transitory starch is substantiated. Confirmatory evidence is had further in the fact that fungi can assimilate tannin, among other aromatic substances,[24] though anything beyond a very low food value is questioned. Such tannin as is here described for the date may, however, not be a food of direct availability, but may be a link in a chain of metastates, or may afford energy in a more direct way, as by oxidation.

The Embryo. An account of the occurrence of tannins in the embryo requires only the general statement that during the earlier stages of development, from pollination on, the embryo, together with the embryo-sac structures, and after fertilization, with the developing endosperm, is richly supplied with tannin. A pronounced general reaction is exhibited by the embryo as late as thirteen weeks after pollination, (Deglet Noor,) when the embryo measures .50 by .42 mm. When full sized, the embryo measures 1.1 mm. by 2.1 mm., an increase which is accomplished in a period (13 weeks) equal to that required to reach the smaller dimensions. During this second period the tannin content of the embryo becomes less and less marked. Nevertheless, until a short time before maturation, (f. 18) a distinct reaction is discoverable, and, though it is diffuse throughout the tissues of the embryo, there is evident a more marked amount of tannin (1) beneath the epidermis of the cotyledon, (2) along the vascular tracts, (3) in the plumule and the tissue beneath it, and (4) in the active zone of cells between the hypocotyl and radicle, which, of course, are both very short and not readily distinguishable. These peculiarities of distribution are clearly connected with the physiological function of the tannin. At maturity, and during the resting condition tannin is quite absent, as stated by Sachs. There are, so far as I have ob-

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[24] Nägeli and Reinke, quoted by Pfeffer, Physiology of Plants, p. 492. M. T. Cook has shown recently that tannin is a poison. It is, of course, obvious that different substances may be meant.

served, no special tannin cells in the embryo during the embryonic or the resting period. In early germination there is according to Sachs' account, a reappearance of tannin, which soon becomes localized. When the extra-seminal portion of the seedling has reached a length of a few cm. the constricted zone of the cotyledon, connecting it with the haustorium, shows a definite tannin reaction. I studied fresh material of the ordinary dates of commerce. Iron chlorid shows, in common with methyl blue, (1) a general reaction throughout all the tissue of the isthmus and (2) a considerable number of special tanniferous cells in the parenchyma in the same region. These react as strongly as the idioplasts. The diameter of the neck is one-half that of the cotyledon just outside the seed, so that the areas of the cross-sections are as 1:4. The reduction of the area does not, however, affect the transporting capacity of this part of the cotyledon, since there is no constriction of the vascular tissues. It would appear therefore that these tannin cells are not to be explained by any nutritive relation. It is aplastic tannin, and, as Sachs believed, plays no active rôle. In the further nutritive phenomena seen in the growth of the haustorium or in the digestion of the endosperm, there is no evidence of tannin. In the cortex of the seedling, chiefly near the growing end, in the region therefore of the hypocotyl, there is observable a slight tannin reaction, but the facts are such as not to lend themselves readily to any explanation. At the close of germination, there are tannin cells distributed throughout the cotyledon, but chiefly in the ventral moiety and in the parenchyma immediately adjacent to the vascular strands. The ventral epidermis contains also a goodly number of tannin cells. The cotyledon is of course moribund at this stage.

In the first foliage leaf a similar behavior is to be seen save that the tannin appears more especially in the parenchyma of the dorsal moiety. In the second foliage leaf, before it has yet emerged from the encasing, cylindrical first leaf, the tannin appears at first in the parenchyma, in scattered cells lying in the middle zone. There is no diffuse

reaction in any case, the tannin being completely confined to the cells in which the secretion at first takes place. The genesis of the tannin may be observed very exactly in the second foliage leaf, at the age indicated. It first appears as minute granules which become larger. These finally coalesce to form a continuous layer about the cell, or, at length, a mass with vacuoles. There is no evidence of a transitory tannin, so that it must be regarded, in the absence of definite evidence to the contrary, as aplastic.

OIL.

No oil appears in the carpel at any time.

In the endosperm oil appears for the first time after about the seventh week following pollination, before the endosperm utriculum is obliterated (f. 8-10). There is a gradual increase, in the form of minute droplets, till about the tenth week, about the time, approximately, when the thickening of the cell walls begins in the endosperm. As already pointed out, this is the time when the tannin appears in connection with the thickening walls. For a short period (f. 26, 26a) at about the time of this phase of development, the oil content is reduced, during which time the seed reaches its definitive form. The oil content then increases again till, in the mature seed, the endosperm cells contain two carbohydrate foods, reserve cellulose and oil. According to du Sablon (1897) the oil constitutes 7-9% of the whole endosperm in the resting seed. I find no evidence of tannin within the resting endosperm save in certain cases due to irregularity of development already noted.

The disappearance of oil and the beginning of cellulose accumulation are without much difficulty explained on the same grounds upon which we may also explain the accumulation of oil as the reserve cellulose approaches a definitive condition. The relation of tannin to these is more obscure, but there is sufficient evidence, already advanced, to regard it as related in some way to the changes which go on during the thickening of the cell walls.

NUTRITIVE RELATIONS OF THE EMBRYO AND ENDOSPERM.

The nutritive relations existing between the germinating seedling and the endosperm have been so frequently investigated that the date has become classic. Under the above caption, it is my purpose, so far as possible, to complete the account by detailing the corresponding relations during the embryonic period. Especial interest attaches to this phase because the growing embryo opposes itself to a growing endosperm, while during germination, the latter is wholly passive.[25] This opposition of two growing and anatomically independent bodies must result in adjustments which may be mechanical merely; or they may be chemical with resulting secondary mechanical adjustments. There must also be a zone where the counter-influences meet each other, which may be termed the tension zone.

Periods I and II.

During the first and second periods, as I have already pointed out, the growth of the embryo is slow. A week after pollination it measures about 25 microns in diameter, and attains at stage III a diameter of only 60-75 microns. It is evident that, in view of the loose anatomical character of the endosperm, and the slow increase of the linear dimensions of the embryo, there is but little mechanical readjustment involved. During the first period, I have found no optical evidence of digestive activity peculiar to the embryo, although, of course, this may obtain. This is apparent from the fact that, at 11 weeks, all the endosperm cells near the embryo are intact (f. 14a), and show no evidence of digestive action in the cell walls. It seems probable therefore, that the embryo and endosperm act as a physiological unit so far as nutrition is concerned. At any rate, the reactions appear to be quite the same in both, and show, e. g., that tannin is abundant in them throughout the first

[25] Pond, 1906.

period. Toward the close of the second period, although there is pressure upon the endosperm surrounding the embryo, there is still no digestion of the cell walls to be noted. There is therefore no breaking down of tissue. During the early part of this period, however, there is to be seen the independent action of the embryo in the unequal distribution of oil in the surrounding endosperm (f. 14). At this moment the accumulation of oil throughout the latter is marked, but much less so in a spherical mass surrounding the embryo. This sphere measures fully 250 microns, so that the embryo exerts an influence to a distance of some 100 microns, more or less, from it. In addition to the small amount of oil in this region, I have noted that my preparations of material of this age all show an accumulation of tannin in the form of droplets (f. 26, 26a). I have not seen it in the same or corresponding region in this form at any subsequent time. The preparations in question were fixed in copper acetate, treated with ferric chlorid and with alkanet, before and after absolute alcohol and chloroform, so that there can be little doubt of the accuracy of the observation. This appearance of tannin is synchronous with the beginning of reserve cellulose formation, when, as already stated, tannin appears in that portion of the endosperm where the thickening of the walls occurs. It may therefore very well be that this tannin is concerned in the nutrition of the embryo, but my material was not sufficient to settle the question and it must in consequence be left open.

To complete the evidence that oil is a nutrient for the embryo at this time it should be noted that it occurs plentifully in the embryo as well as in the endosperm. The appearance of the oil in the embryo differs from that in the endosperm. In this, when the oil first appears, it occurs in extremely minute droplets, which collect in a zone about the nucleus. This is especially well shown by the pavement endosperm cells lining the cavity (f. 9). In the embryo, however, this relation is not to be seen. Here the oil occurs anywhere in the cytoplasm, as depicted by Sachs in the epithelium of the growing haustorium.

How the distribution of oil at this time is to be understood is not immediately clear. There appear to be two alternatives. The comparative absence of oil in the vicinity of the embryo may be due to the immediate absorption by the embryo of most of the material which would, in this region, be turned into oil; or to the movement of the oil, after it has been laid down, toward the embryo. In the latter case, we may assume a lipase to account for digestion, or we may assume that the fatty material moves in the form of minute globules, as Sachs believed. The occurrence of droplets of oil between the embryo and the endosperm was regarded by Sachs as evidence that during germination the immediate entrance of this substance into the embryo is accomplished without change of its molecule. I myself have observed the occurrence of large droplets of oil in the same situation during both the embryonic period and germination. Figure 15 is an example of the former, which, however, is explainable otherwise. Assuming that the oil actually occurs here normally, it seems probable that it is due to the accumulation of small droplets from the disintegration of the cells in which it occurs. (zone *a*, f. 15). This would readily be brought about if the digestion of the oil at this time did not proceed as rapidly as the breaking down of the cell walls.

The large masses of oil in the space between the haustorium and the compressed endosperm during germination on the other hand, I believe to be purely accidental, and is due to the transposition of the oil by the knife-blade during sectioning. There is, furthermore, convincing evidence that during the major part of the third period of embryogeny, as well as during germination, that there is an actual digestion of the oil, resulting in water-soluble substances, and it is therefore entirely probable that the same is true of the still earlier periods during which oil plays a part in the nutrition of the embryo.

Period III.

The final period of development is of peculiar interest in that it leads to the articulation of the two distinct physio-

logical periods, that immediately before and that following, germination. Of much information regarding the latter we are already in possession, chiefly from the work of Newcombe and of Pond, following that of Sachs, Reiss, du Sablon, and others.

The period in question involves a marked mechanical adjustment of the embryo and endosperm. At first the embryo is spherical in form (f. 14) becoming, at 13 weeks (f. 16) broadly ovate-conical, with the base against the micropyle. Scarcely any change in shape is now to be noted for two weeks, after which, however, the growth is rapid. Within another month, the definitive form and almost full size are attained. (f.18). This, especially, is the period of mechanical adjustment, for it is now that the endosperm is also growing rapidly, and that the secondary thickening of the cell walls, to form the reserve cellulose, takes place. But during this time approximately, the radial growth of the endosperm is relatively much more rapid than that of the embryo, and there arises as a result a cavity in the endosperm, opposite the cotyledonary end of the embryo, which is at length nearly obliterated (f. 15-19). For the purpose of enabling the reader to follow the changes which ensue during this period, I have chosen six stages of development, and represented these in figures 14a-19, inclusive, to which the following descriptive notes may be applied.

Figure 14a.—Zone *a*. Cells show no disintegration, but are large, with a relatively small amount of protoplasm, and reduced oil content. No blue reaction in the cell-walls with iodine.—Zone *a* becomes differentiated into zones *a* and *a′*.—Zone *b*. Small endosperm cells with divisions taking place approximately parallel to the integument. This characterizes also the peripheral endosperm, beneath the inner integument.—Zone *b′*. Thin-walled endosperm cells, larger than in zone *b*.

Figure 15.—Zone *a*. Cells with collapsed walls, empty or with minute protoplasmic content and a droplet of oil. *The walls react blue to iodine.* In the space between *a* and the embryo, oil accumulates.—Zone *a′*. Uncollapsed cells within the digestion zone. The outermost show no change. The innermost show a more or less disintegrated

condition in the protoplasm. *The walls react blue to iodine.* The small quantity of oil becomes more evident in the inner cells as droplets removed from the protoplasm.—Zone *b*–*b'*. Thin-walled endosperm cells in which cell divisions occur, more especially in the layer of cells between *b* and *b'*. Tension zone, contributing cells to zone *a'* and to zone *c*.—Zone *c*. Endosperm in which the thickening of the cell walls has progressed. The limit between zone *b* and zone *c* is not a sharp one. (Zone *c'* is not visible, but has begun in the interior of the endosperm. cf. fig. 26a.)—Zone *b'* now becomes compressed between the advancing thickening endosperm, zone *c*.

Figs. 16, 17 and 18.—Zone *a*. Cell walls entirely collapsed, reacting blue with iodine. All the contents except minute droplets of oil have entirely disappeared.—Zone *a'*. Cell walls not collapsed, reacting blue with iodine. The cells nearer the embryo have no other content that each a single large droplet of oil. The outermost have more or less protein content.—Zone *b*. Unaltered endosperm cells, the inner of which are subject to digestion. The outermost cells of this layer are represented by zone *b'*.—The tension zone becomes narrowed down to zone *b'*, of cells undergoing division, and shearing between the growing endosperm and zone *b*.—Zone *c*. Endosperm with thickening cell walls, giving a tannin reaction only in the lumen. Zone *c'*. Definitively thickened endosperm cell walls. Deep tannin reaction in the cel-walls in the younger stages but disappearing in the older ones.—The zones now become compressed and the distinctions less marked or nearly obliterated.

Figure 19.—Zone *a*. Crushed and compressed cells reacting blue walls and contents. This zone is separable into two zones by the successful application of iodine and sulfuric acid. This treatment produces a deep blue reaction which quickly fades away, and causes swelling and dissolution. These proceed differently however in the two layers a and *a'* figure 20. The compressed cell walls of layer *a* swell and dissolve as a whole; those of *a'* first expand and assume their original form previous to swelling, indicating that they are not so far disorganized by digestion as those of *a*. The compressed cells contain numerous minute oil droplets; the less compressed (*a'*, f. 20) large droplets.—Zone *b'*. Tension zone. The cells are partially thickened, less toward the embryo, more toward the outside. They are secondarily divided by transverse walls. They contain protoplasm and oil, the latter in large drops, the protoplasm more or less disorganized.—Zone *c*. Definitively thickened and characteristic endosperm cells.

It may here be noted that the distribution of oil in the compressed zones is not as figured by Sachs (his figure 4, pl. 9, 1862), who represented large drops as occurring anywhere within the compressed layer. The treatment with sulfuric acid demonstrates this very beautifully, as the droplets are displayed in their relative positions as the wall material swells. This is true of the whole period of germination.

We are now in a position to present in summary form the changes which occur.

Zone of Digestion. At first a spherical mass of intact cells (*a,* figure 14a) this becomes differentiated into two zones (a, a′, figure 15) due to the collapsing of the walls in zone a accompanied by the digestion of their contents oil and protein. We have therefore to note (1) the cause for the collapsing of the cells which is found in the digestion of the primary walls; (2) the disappearance of the contents, due to the digestion of the oil and protein.

Digestion of primary cell walls. Sachs (1862) believed that the primary membrane is not digested during germination, but that the growing haustorium pushes the exhausted and crushed cells before it. Reiss (1889) extended Sachs' observation of the endosperm of *Chamaerops humilis.* I have found no evidence to the contrary after a stage of germination roughly indicated by an embryo length of 2 cm., at which stage the haustorium has attained the form of a sphere (f. 24), so that there exists no lack of harmony between my results on germination and those of Sachs and Reiss, which have been generally accepted.

Newcombe (1899) extracted a ferment capable of digesting the *whole* of the endosperm cell wall. This result is of interest in that there is yet advanced no optical evidence that the primary membrane is actually digested in the date. Newcombe's results appear to indicate that there are two ferments extractable together,[26] one capable of acting on the primary wall, the other on the reserve cellulose. The former may be present during germination in such small amounts that the result of its action on the primary wall is inappreciable to the eye, but that, during the prolonged period of experimentation made use of by Newcombe, it had time to act. Probability is lent to this interpretation by the work of Green.

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[26] The contrary view that a single ferment isolated by Newcombe is capable of digesting both primary walls and the reserve cellulose is negatived by the positive evidence that such digestion does not take place during germination; though the logical possibility remains that it takes too long or that it is inhibited in some way.

Green (1899, p. 97) in 1887 studied the progress of digestion in the germinating seedling of *Livistona humilis,* which is quite similar structurally to the date. After two months germination at which time the endosperm was about half absorbed, Green found that the "inner zone"[27] of the digested portion of the endosperm gave a blue reaction with iodine, but failed to react with chlor-zinc-iodine. Green concluded that under the action of a cytatic ferment the cell wall (primary membrane) was changed. This form of digestion in the primary membrane Sachs did not see in the date, and, as far as microchemical methods may tell us, it appears that he was right. During the embryonic period, however, it is otherwise. My observations show that soon after a stage represented by figures 14a, 26, when the adjustments between the embryo and the cell wall of the endosperm are purely mechanical, a substance is secreted, presumably by the embryo, which acts upon the nearest cells, attacking the primary membrane and changing it into an amyloid, as I may call it for the present. The reaction indicative of this conclusion is to be seen clearly at a stage represented by figure 15, in which a column of cells (*a,* in the figure) opposite the apex of the embryo all show a distinct and characteristic blue coloring. The walls themselves in zone *a* appear wavy and more or less crumpled by lateral pressure of the cells of zone *a'*, and show every appearance of undergoing some sort of change. The blue reaction is shown also by the walls of the uncrushed cells (a', f. 15) flanking the column of crushed cells (a, f. 15). This behavior in the presence of iodine indicates clearly that the membranes are undergoing a chemical change, hydrolytic in nature, similar to that observed by Green during germination in *Livistona.*

That this digestion is such in fact is shown also by the behavior of the crushed cell walls (a, f. 20) nearer the embryo as compared with those in a layer further removed (a', f. 20). The former are directly dissolved by sulfuric acid while the latter spread out and take their normal form before dissolution. The exceedingly tenuous character of

[27] I understand this to mean that next the haustorium.

the crushed walls next the embryo, as compared with the thicker condition further away also points to the same conclusion. It may finally be noted that the earliest mechanical disruption of cells is not due to the pressure of the embryo upon the contiguous endosperm cells, but of the cells of zone *a'* upon those of *a* (f. 15). It takes place previous to the secondary thickening of the endosperm walls by the deposition of reserve cellulose. As the embryo grows, the amount of tissue thus affected increases markedly (f. 16, 17) until a time of maximum activity is reached, which happens about eighteen weeks after pollination, at a stage represented approximately by figure 18. The reaction obtained at this time indicates that zone *a'* (f. 18) may reach quite near to the outer limit of zone b. My preparations show further that the blue reaction is not always confined to the cell wall, but may frequently fill the lumen, indicating the presence of a soluble or colloidal substance here.[28] On approach to maturity, the relative thickness of the layer of cells affected becomes reduced, and at the time of ripening, occupies the zone *a* shown in figure 19. I have verified this by control with commercial dates, which give the same reaction.

During the early stages of germination I have found only a partial reaction and this is confined to the endosperm in contact with the older part of the growing haustorium (f. 24, 25). In a stage corresponding to figure 25, the blue reaction appeared along the flanks and around the end of the cotyledon, but in the latter position it was very thin and discontinuous. In a somewhat older condition, as in figure 24, the reaction was to be seen only along the sides of the haustorium, corresponding to the extent of the sides of the cotyledon seen in figure 25. It seems probable therefore that the change of the primary membranes into an amyloid is chiefly an embryonic phenomenon, and ceases during or

[28] I have found some evidence that a small amount of starch in the form of minute granules occurs. These are scattered between the crushed cell walls. The amount seen however is very small, and I have not always been certain of its presence. This uncertainty may be due to its disappearance and reappearance.

perhaps previous to, the early stages of germination. It is a fact of very great interest however that this form of digestion, which in *Livistona* takes place during the course of germination, occurs in the date only during the embryonic period. I therefore conclude that there is secreted by the growing embryo during the period from the age of about 10 weeks till maturity an enzyme, probably a cytase, which causes the hydrolysis of the primary walls of the developing endosperm in the region of the embryo. It is to be further inferred that this digestion leads to the formation of a sugar, in which form it is absorbed by the embryo.

Digestion of oil and protein—(a) During embryogeny.—The separation of the zone of digestion into two sub-zones, a and a′ in the figures, of collapsed cells within, next the embryo, and uncollapsed cells further out enables us to describe the course of events relative to oil and protein with reference to the mechanical condition of the cell walls.

At the beginning of the period the zone (*a*, f. 14a) is undifferentiated within itself. The cells contain only small amounts of oil (f. 14). Before the digestion of the cell walls, as already described, sets in, the amount of oil increases. The digestion of a column of cells in front of the embryo (*a*, f. 15) is accompanied by the disappearance of the protoplasm and the isolation of the oil so that small droplets are seen within the strand of crushed and empty cells. Assuming for the moment that there is an actual digestion of this oil, it must be slow, since there collect large droplets in the space next the embryo. That the breaking down of the cells at this time should proceed more rapidly than the undoubtedly slow digestion of oil may be understood by the fact that the volume of these cells is relatively much greater than if their compression were due merely to the pressure exerted by the embryo. In point of fact, this pressure is exerted by the adjacent undigested cells in the direction indicated by the arrow points in the figure. Now begins the rapid growth of the embryo, accompanied by the compression of the cells adjacent to its surface (f. 16), but the growth of the whole endosperm in radial direction, as indi-

cated by the arrow point at the bottom of the figure, results in the formation of a pit in front of the cotyledon. This is obliterated only with complete maturation (f. 19). The crushed layer of cells lines this pit, their crowding, which does not amount to compression, still caused by the adjacent tissues. The later progress of events consists in the amplification of these relations till a stage represented by figure 18, is reached, followed by a reduction during maturation.

Observation during the whole of the period shows that the distribution of protein and oil in these tissues may be briefly summarized.

The outermost of the uncollapsed cells (zone *a'*) contain both their oil and protoplasm in practically an unchanged condition. Passing toward the embryo, the protoplasm shows evidence of disintegration and obvious reduction in amount till the inner layer of uncollapsed cells is reached, where only the oil remains usually as a single drop in each cell. Within the crushed layer, these oil drops are quickly reduced in size (f. 18a and 20).

(b) During germination.—The zones[29] of digestion surrounding the growing haustorium are as follows, beginning at the plane of contact with the embryo (f. 24; text figure B). The thickness of the zones depends upon the position relative to the haustorium, being thickest opposite the rapidly advancing edges:

a. **Crushed cells derived from embryonic period, reacting blue with iodine. This zone is discontinuous and is derived from the resting condition.**

b. **A zone of compressed cells, of primary membranes only, about 15 microns thick. The oil is in minute droplets. (Sachs' layer 1).**

c. **A zone in which the oil is in a large single drop in each cell; the protoplasm has disappeared. The reserve cellulose is less in amount proceeding toward the embryo and the cells are more compressed (text figure b). (Inner part of Sachs' middle layer.)**

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[29] **The reader will not infer that these zones are sharply delimited, any more so than the others which I have described. Nevertheless, they represent an objective reality.**

d. A zone from which the protoplasm is disappearing, and in consequence, the oil becomes agglomerated. The reserve cellulose begins to undergo change. This zone takes up Bismarck Brown more readily than elsewhere. (Outer part of Sachs' middle zone.)

e. Unchanged endosperm. (Sachs' corresponding layer.)

This somewhat sharper analysis than that of Sachs has for its purpose to show that the agglomeration of the oil is coincident with the disappearance of the protoplasm, and that the disappearance of oil begins when the cell-walls are reduced to the primary membranes. This harmonizes with du Sablon's conclusion that the amount of oil in the undigested endosperm remains the same (7-9%) during germination, and that "on peut donc dire que la matiére grasse de l'albumen n'est absorbée par le cotyledon qu'au fur et à mesure de la digestion des parois mêmes des cellules de l'albumen" (1899, p. 396). It may be observed however that the relative amount of oil in zone *c* is greater than elsewhere, because of the reduction in the amount of both the protein and reserve cellulose. The comparison of the behavior of oil and protein during embryogeny and during germination shows further that the facts are identical, while the cessation of growth in the endosperm and the presence of reserve cellulose remove one condition, the presence of the tension zone, and impose another, the necessity of digesting the reserve cellulose, a different material from any attacked by the embryo previous to the resting stage.

The conclusion seems obvious, that during the embryonic period the oil is digested and, before absorption by the embryo, changed into water-soluble substances. This conclusion must be extended to the period of germination. Sachs believed to the contrary, for the reason, before stated, that oil occurs in large quantities in the compressed layer and in the space between this and the epithelium of the haustorium, and because the immediately underlying layer of epithelium also contains oil-droplets[30] (1862, p. 250). The presence of

[30] Reed (1904) in his studies of the cytology of the epithelium of the date appears to have overlooked the important modifications in the appearance of the cytoplasm, and possibly of the nucleus, which

only a small amount of oil in the embryo was noted by du Sablon, but he observed also notable quantities of sugar (*l. c.* p. 398).

I have already explained the presence of oil in the space between the embryo and endosperm as due to accident in the cutting of freehand sections. It would, I think, be apparent to anyone at a glance that this explanation is the true one. The presence of oil in the embryo is explainable on the ground that the materials derived from the endosperm which are not immediately used by the growing embryo are reformed into oil and starch. This explanation was applied by Sachs to starch. It is evident that the presence of a "notable proportion of sugar" was regarded by du Sablon as the part equivalent of the oil. My own contention is that the oil found in the embryo is derived from the water-soluble materials, derived from digestion of oil, and which, upon entrance into the epithelium of the embryo, are not removed by use, either there or elsewhere. In support of this view the following additional evidence is advanced:

In a germinating seedling, the extra-seminal portion of which was two millimeters long (figure 25) the digestion of oil in the endosperm *in front of* the end of the cotyledon, which alone at this stage is haustorial in nature, was proceeding. There was, however, no trace of oil to be found in the epithelium of the cotyledon in this region, but only in that flanking the cotyledon, opposite endosperm cells in which no digestion is going on. The distribution of oil is shown in figure 25 by the stippling. In the same general region, but in the parenchyma beneath the epithelium there is starch to be found. In a somewhat older seedling (f. 24) there was no oil to be found in the epithelium at all, although

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must ensue upon the accumulation and reduction of oil, which doubtless occur during relatively short periods of time. In examining living material, Reed further speaks of numerous large granules all of which react to protein reagents, but does not indicate that other reagents were employed. The subject of Reed's study is admittedly peculiarly difficult, and for this reason it is all the more important that the appearances of the cells in question should be explained only after taking all the substances occurring in them into consideration.

the region of digestion had by this time spread along the flanks of the cotyledon. In two seedlings in which the first foliage leaf was 3.5 cm. long, in which therefore the haustorium was quite large and nearly fully developed, I found the oil very unevenly distributed. In most of the epithelium cells there was none. A comparatively few cells in isolated groups showed a rich oil content. In no case was there any oil in the parenchyma below. In a similar seedling after a week's exposure to ethyl nitrite, there was a very large oil content, the epithelial cells being quite replete,[31] so that one could hardly observe the remaining contents. Scarcely a cell varied from this condition. Similar results were obtained in a younger stage when the first foliage leaf was just emerging.

The conclusion is therefore drawn that the disappearance of oil during the development of the embryo and during germination is due to the same cause, namely, digestion. This digestion results in the formation of water-soluble substances, and not of an emulsion. The reappearance of oil in the embryo is due to its reformation from these substances in consequence of the failure of the embryo to consume them as rapidly as formed.

The particular behavior of the protein I have not especially considered, beyond to note the time of its disappearance from the cell relative to that of the oil. Sachs believed that "albumen" enters the embryo "as such." It is apparent that this material must undergo proteolysis if we accept evidence of the same character as that advanced with regard to oil and cellulose.

The Tension Zone.—The limits of the various zones are not sharp, so that, in making diagrammatic representations there is necessary some compromise as a sacrifice to confusing detail. In the first stage considered (f. 14a) the tension zone is little more than a vague layer of cells (*b*) somewhat crowded between the digestion zone *a* and the thin

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[31] A large increase in the starch content of the parenchyma beneath the epithelium was also noted.

walled but growing endosperm, *b'*. With a slight advance in age, it becomes evident that the inner part of zone *b* is giving cells to be digested, and these then become part of zone *a'*. At the same time, zone *b'* becomes narrowed down by the advancing zone *c* (f. 16), endosperm in which reserve cellulose is being laid down. This results in crowding the cells in which divisions are taking place into a narrower compass, and they occupy a region whose position is approximately represented by the narrow cross-hatched line between *b* and *b'*. It will be observed that the actual histological results of tension are seen at first in zone *b* while in later stages they pass over into zone *b'*, as represented in the diagrams. That is to say, thin-walled endosperm cells entirely disappear, but are represented by cells which are secondarily transversely divided and at the same time somewhat secondarily thickened. Zone *c'* disappears because tannin does so. This fact is represented in the diagrams by the line of demarkation overtaking, and becoming coincident with, the inner limit of zone *c'*.

In endeavoring to understand clearly the course of events it is necessary to keep in mind the directions of growth (1) of the endospermic plug tissue overlying the base of the embryo, and which thickens independently of the main mass of endosperm; (2) of the embryo, which is growing in thickness and length, on the one hand; and (3) of the main body of endosperm, expanding radially in all directions toward the inner integument as a limit. The enlarging endosperm cells, namely those in the interior, push against the dividing endosperm cells, which are peripherally placed. These occupy a position, therefore, between the inner integument and the inner part of the endosperm, the cells of which are enlorgating radially. These thin walled, dividing cells constitute a tension zone. But in the region of the embryo, the digestive zone takes the place of the inner integument, so that the tension zone, where the secondary division therefore are taking place, dips inwardly. The plug tissue however is also in division, and this independent disc of tension tissue meets and merges with the other at the place

where it dips inwardly. The place of juncture becomes more and more obvious with age till the completion of secondary cell-wall thickening, when it remains visible on account of the irregularly matched cells. This is a plane of weakness where, during the initial stage of germination, the endosperm plug is released.

The constant advance of the definitive endosperm opposed to the growing embryo results in the reduction of the zone in question. I have already pointed out that the secondary divisions in the endosperm cells which characterize it are always parallel to the integuments. They are therefore at right angles to the axis of the embryo. If these are taken as the criterion of the zone, it is seen that it is at last reduced to a thin layer of cells (*b'*, f. 19, 20), which are able neither to take on the definitive character of endosperm cells, nor entirely to resist the influence of the embryo. This is shown by the partial thickening of the walls, and in the partially digested condition of the contents. The shearing caused by the opposed directions of growth is also to be seen in the oblique and more or less distorted forms of the cells (f. 20, 21).

Tannin in the zones of digestion.—It has been shown that during the third period of development tannin figures prominently in the endosperm in connection with the laying down of reserve cellulose. It has been pointed out, however, that the reaction is not confined to the cells alone which are active in this regard, that, in a word, it occurs everywhere in the endosperm, the statement being intended to include the zones of digestion. For example, ethyl nitrite material thirteen weeks after pollination showed that the tannin in the tension tissue and in the digestion zones proper is in marked amount, as it is also in the embryo. This is true of younger stages, and of more advanced ones until the condition is reached represented by figure 18, when, along with the general reduction of tannin throughout the endosperm, the amount in the digestive zones is also reduced. This disappearance is not synchronous with that in the embryo, in which tannin is discoverable for a longer period. It must

also be added that the tannin in the cells in these zones, is not confined to the cell walls as it is elsewhere in the endosperm. This, with the earlier disappearance of tannin in them than in the embryo, may be taken to indicate that the tannin here is concerned chiefly with the embryo.

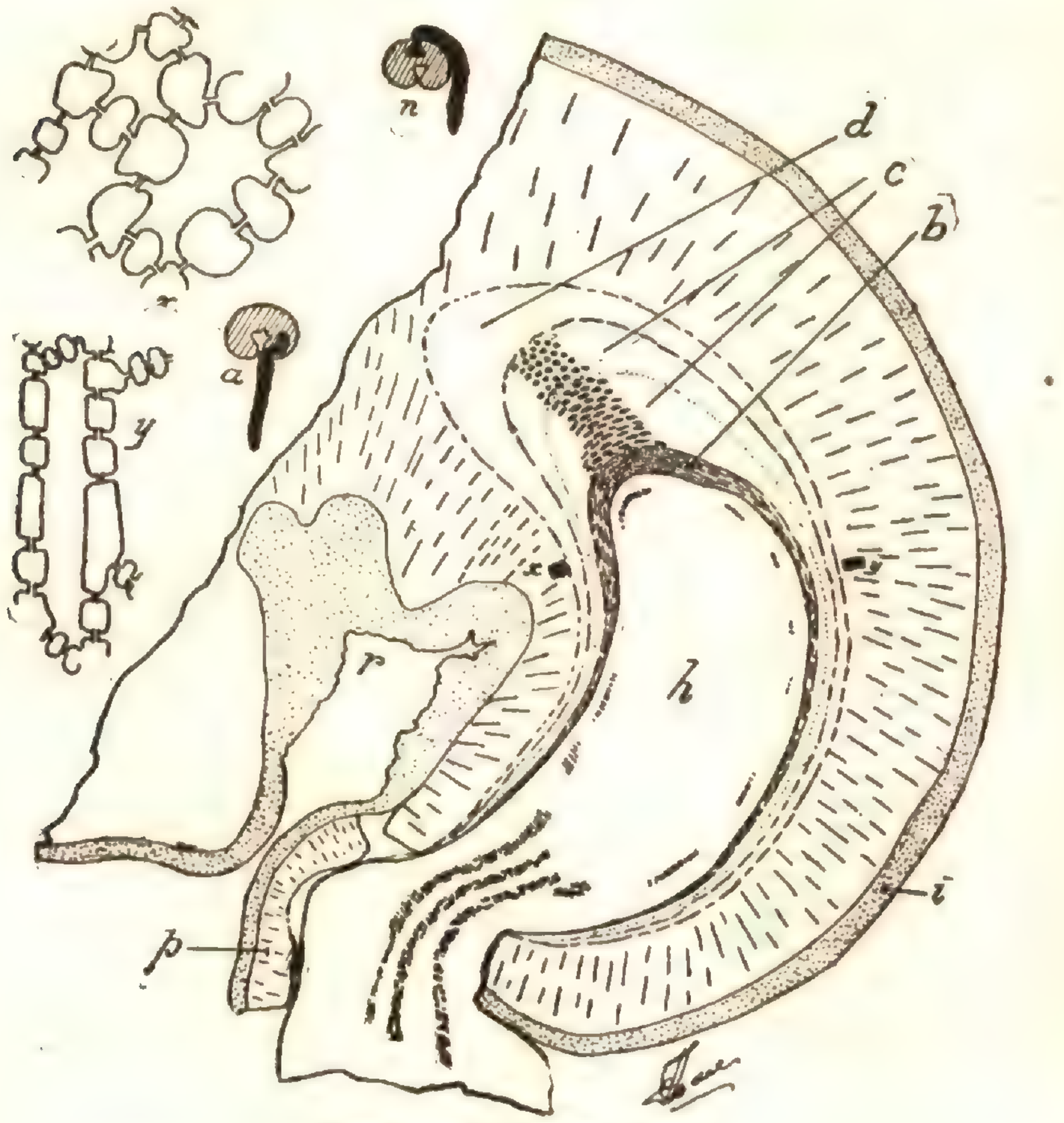
Digestion of Reserve Cellulose.—As reserve cellulose plays, if any, a very inconsiderable part in the nutrition of the embryo and because it has been studied, first by Sachs, and very carefully by Reiss (1889), both histologically and chemically, it has been no part of my purpose to concern myself with it. I can find during embryogeny no evidence that there is any digestion of reserve cellulose, even during the short period when this may be suspected to occur. On the contrary, as I have already pointed out, the process of cell wall thickening proceeds inwardly toward the embryo until the resting condition is entered. One point of disagreement, however, as between Sachs and Reiss has been looked into by me, namely, the presence or absence of the optical evidence of the primary membrane in the definitive endosperm cells. Sachs says: ". . . die primären Zellhäute sind leicht als doppelte conturirte Lamellen zwischen den Verdickungschichten zu erkennen"[32] (1862, p. 242). This Reiss denies categorically, thus: "Die Wände lassen keinerlei Schichtung, auch keine Mittellamelle sichtbar hervor." The fact appears to be that the observation is not as easy as Sachs' wording would lead one to expect. While not everywhere visible with equal ease, however, there is no very great difficulty in determining the substantial correctness of his statement. As for the manner of digestion of the reverse cellulose, I am able to verify Reiss' account.

Origin of the Ferments.

Sachs believed that the digestion of the endosperm in the date is due to a ferment secreted by the endosperm. Were this not so it would be difficult to explain the remarkable coincidence that the softening of the endosperm exactly cor-

[32] Quoted by Reiss.

responds to the growth of the haustorium. "Dieser Umstand macht es eher wahrscheinlich, dass die Epithel einen Stoff an die nächsten Endospermzellen abgiebt, der die Lösung des Zellstoffs bewirkt."



B. PHOENIX DACTYLIFERA.

Transverse section through a germinating seed in which the embryo has formed in an abnormal position.—*h*, Haustorium. *i*, Integuments. *p*, Endosperm plug which caps the embryo during the resting stage. *r*, Raphe, partly torn out. *x*, *y*, Portions of endosperm the cells of which are represented on a larger scale (the same for both) at the left. The relative position of this abnormally placed embryo and of a normally placed one is shown at *n* and *a*.

Nevertheless, Sachs' conclusion was not accepted without demur. Grüss (1896) especially thought he had proved to the contrary, though his evidence is not convincing. Newcombe (1899) showed that a cytohydrolytic enzyme could

be extracted from the endosperm, and he thought that it was present "in the softened layer of endosperm against the cotyledon" but it is clear from the context that he did not believe the enzyme to originate in the endosperm but in the cotyledon. The point however being moot, Pond (1906) showed conclusively that cytase does not originate in the endosperm.

Sachs' reasoning is thus justified. It is a well-known fact, however, that the outline of the zone of digestion is not parallel to the surface of the growing haustorium, and while this does not vitiate the reasoning it calls for explanation. Figure 25 shows that the digestion begins at the end of the cotyledon, where the endosperm cells are isodiametric. It is clear that there is a localization of enzyme secretion in this region of the cotyledon. The movement of the ferments into the endosperm from this on must be due either to the anatomical conditions in the endosperm, or to a continuation of the localization of more active ferment secretion. If one follows the movement of the haustorium, one sees that it is not related to the shape or position of the cells of the endosperm (text f. B). Its more rapid growth may be parallel to or athwart the longitudinal axes of the cells, and is not always the same in amount and direction in the regions where the cells are approximately isodiametric. There is no evidence, moreover, that there is any more usable passage-way through the endosperm by virtue of thinner walls or more pores. We must conclude, therefore, that the shape of the digestion cavity is in large part determined by the localization of greater activity of ferment secretion in the haustorium. We are urged to the conclusion, further, that the greater secretion of ferments is at first at the end of the cotyledon, but that it becomes relatively less active, the scene of greater activity passing over to the sides of the haustorium. In this connection, the finding of a seed in which the embryo had suffered abnormal displacement, so that it lay in a position almost antipodal to the normal one. is of interest. It was discovered only after it had been germinating for some time (f. B).

The endosperm was normal in respect to its histological characters, its cells being arranged in the usual way. Presumably there was some adjustment quite near the embryo, and there is evidence for this in the configuration of the cells in the plug. Both the region of greatest growth in the haustorium and the area of most active secretion are opposite to the greatest thickness of endosperm to be digested. We cannot in this case say that this part of the haustorium is the end or the side, but it appears conclusive that the character of the endosperm cells has nothing to do with the direction of growth of the haustorium. We may, indeed, say that the behavior of the haustorium is adaptive, and that this organ adjusts its behavior adaptively when the normal conditions are disturbed, until a more satisfactory explanation is to be had.

SUMMARY AND CONCLUSIONS.

The more important features of the foregoing account may be summarized as follows:

1. The development of the embryo, seed and pericarp with reference to nutrition have been followed from the anatomical-physiological point of view.

The embryological history has been articulated with the period of germination, and the continuity or discontinuity of the various processes involved in the digestion of the endosperm has been determined.

2. Before the time of pollination, the antipodal cells, and for some time thereafter, a tapetum are active agents in the nutrition of the embryo-sac. This first digests the small nucellus; it then becomes active at the antipodal pole, and forms a group of digestive pockets around the degenerating antipodal apparatus which ultimately form a single cul-de-sac, the function of which is to digest ovular (chalazal) tissue and to receive nutrients from the raphe. The chalaza in the mature seed is not determined by the configuration of the integuments (as is frequently the case), but by the manner of development of the endosperm cul-de-sac, which may in turn be related to the main path along which food materials pass in the raphe.

3. The earlier phase of development of the seed is marked by a longitudinal distortion which results in altering the position of the embryo, swinging it through an arc of 90°. This feature is common among the Palmae.

4. During the first three months following pollination the growth of the embryo is very slow. This period is devoted to the growth of the tissues of the ovule. The following period is marked especially by the development of the embryo and the laying down of reserve cellulose by the thickening of the walls of the endosperm cells.

5. Starch has been found to play only a brief rôle in the basal portion of the carpel and in the ovule. It is found in small and continually reduced quantities until the embryo is 6–7 weeks old. At this time traces only are found in the integument between the micropyle and the funicle. Subsequently none is found, till the embryo is about 17 weeks old, when it may appear in the radicle and in the cotyledon. Its appearance in any position may be taken as indicating a temporary reduction of growth activity in that place. This appears also to be true both of starch and of oil in the early stages following germination.

6. Certain tannins are important quantitatively and in their nutritive relations. I have distinguished for convenience between aplastic and plastic tannin. Aplastic tannin appears in particular cells and remains there permanently. Plastic tannin undergoes translocation, is consumed and disappears. This is taken as evidence of its nutritive rôle.

(a) Aplastic tannin in the carpel occurs in the hypodermis and in the idioplasts. The latter form a well marked sub-hypodermal zone which begins to be laid down at about the time of pollination. Tannin as such does not migrate into these cells, nor into the hypodermal tannin cells. There is therefore no evidence that during ripening there is a segregation of tannin, as reported for certain other fruits.

During ripening the tannin in these cells, as indeed all the aplastic tannin, becomes insoluble, and hence tasteless. There is no evidence forthcoming that this tannin is a glucoside and is oxidized, as tentatively held by Slade[33] and for which a slight amount of evidence has been mentioned by Vinson. Slade's view, however, is possibly applicable to the tannin described in the endosperm, and it would thus be a source of energy (*vide infra*).

The idioplasts form a morphological layer of cells which bends toward the ovule in the sutural region, but is here discontinuous. There is evidence here however of the presence of a small amount of tannin of translocation which becomes more and more marked in quantity toward the base of the carpel and in the tissues subjacent to the ovule, or young seed.

Aplastic tannin also occurs in a comparatively few, usually elongated, elements scattered throughout the inner mesocarp, and in a few cells also of the endocarp.

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[33] See Vinson (1907).

(b) In the ovule all the tannin is at first plastic. Its distribution is such as to indicate clearly that it is concerned in the nutrition of the embryo-sac. The period following fertilization is characterized, on the anatomical side, by the torsion of the ovule, and, on the physiological side, by the rapid growth of the chalazal end of the embryo-sac into the tanniferous tissue of the raphe. The whole of the ovular tissues, properly speaking, now contain tannin, and there seems to be little doubt that it is given over to the growing endosperm. However, there begins the individualization of cells as tannin-cells, which appear at first in the raphe and about the outer limits of the chalazal tissue. Later they appear also in the integuments, with the exception of the epidermis and an irregular hypodermal layer. This tannin is like that in the carpel, as it remains permanently in the special cells. Definitively, the integuments and raphe are completely loaded with insoluble tannin, with the exceptions noted. The precise relation between the plastic tannin and that which gradually appears in the manner described is not clear. They may be quite distinct, or the aplastic tannin may represent unused or unusable tannin side-tracked to accumulate as waste.

(c) In the endosperm there is from the time of fertilization till about the 19th week a very large amount of tannin. Toward the period between the obliteration of the endosperm utriculum and the secretion of reserve cellulose, there is a reduction of tannin, but when the reserve cellulose begins to be laid down there is at once a large increase in the amount of tannin within the same area. This tannin is to be found in the walls as well as in the lumen, and not as artefact. With the maturation of the endosperm, the tannin disappears in a manner to preclude the explanation that it is thrown out of the endosperm as waste.

Whether this disappearance is caused by oxidation, as is known to occur in the apple[34], or by its incorporation into a substance (possibly the reserve cellulose) with a more complex molecule, the evidence does not help us to decide.

(d) Tannin appears in the embryo in larger or smaller quantity throughout the whole of the time of development. It seems to be a principal nutrient during the earlier phase, for which there is the positive evidence that tannin is to be found in the sphere of embryonic influence as droplets, when it is seen nowhere else in similar quantity or appearance. None is found in the resting embryo. Aplastic tannin occurs in certain situations in the seedling, as observed by Sachs, and rightly interpreted by him, so far as we can yet see, as waste.

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[34] Lindet, cited by Kastle, 1910.

7. Oil. There is no oil in the carpel, integuments or raphe at any time.

Oil first appears in the endosperm during the process of ingrowth leading to the obliteration of the utriculum. The amount increases steadily until the utriculum is obliterated, or shortly thereafter. There is then a reduction in amount until the secretion of reserve cellulose has advanced somewhat, at a time approximately between that represented by figure 26a and that by figure 27. It then accumulates till the resting condition is reached. During germination it is digested before passing into the embryo.

In the embryo, oil has been found soon after it appears in the endosperm. There continues throughout the embryonic period a digestion of oil in the endosperm, which is carried on in the same manner as during germination. Its appearance in any part of the embryo is correlated with the relative cessation of activity in that part.

8. Digestion of the primary cell walls near the embryo occurs. This begins approximately between the stages represented by figures 14a and 15. The evidence for this conclusion is to be found in the change, effected in the cell walls, in a column of tissue opposite the cotyledonary pole of the embryo. Here the walls react to rather strong iodine (KI–I) by becoming blue. This material appears to afford but a relatively small amount of food material. The same form of digestion has been described by Green in *Livistona* during germination. In the date, however, it ceases at the entrance of the embryo upon the resting stage, or, at the latest, very soon after germination begins. During the embryonic period therefore, the primary membranes are digested, and this, as recorded by Sachs, does not occur during germination. The middle lamella appears to persist.

9. As shown by Pond, the digestive ferments are secreted by the embryo entirely. It is here shown that the secretion is localized, and is not equally active throughout the superficies of the embryo or haustorium. But this does not wholly explain the behavior of the movement of the haustorium through the endosperm.

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EXPLANATION OF PLATES.

Plate 15.—*Phoenix dactylifera.* 1, Portion of an ovule at about the time of pollination, showing the embryo-sac, tapetum and nucellus, just prior to the disintegration of the last named. 2, Ovule with the contiguous carpellary tissue to show the stylar canal leading to the pollen-tube guiding tissue. The occurrence of tannin in speciaquantity in this tissue in front of the micropyle is indicated by stippling, as also in the embryo-sac, tapetum and inner integument, and chalazal tissue. ' 2a, 2b, Transverse section through the ovule to show the pollen tube canal, single above and double at the funicle. 2 c–e, Transverse sections to show the stylar canal at different levels. The dots in the hypoderm represent tannin; stone cells cross-hatched. Raphide cells are seen in 2 d. 3, Embryo-sac and tapetum at this time. The remains of the nucellus are seen as a cap over the end of the embryo-sac. Digestion of the chalaza is proceeding rapidly. 4, Pit formed of contiguous thick walls of adjacent cells; the antipodal cells within this pit before degeneration of the basal cell is apparent. 4a, The basal and middle cells degenerated. 5, Ovule with one-celled embryo. Multinucleate parietal endosperm. Its activity beyond the antipodal apparatus is evident. 6, Ovule 7–8 weeks after pollination. Distribution of tannin (the more densely reacting tissues) shown by the dots; starch by cross-hatching. Deglet Noor. 7, Ovule, Deglet Noor, 8-9 weeks after pollination. The post-chalazal growth of the endosperm is seen, together with the general torsion of the ovule in the direction indicated by the arrow points. Tannin indicated by the dots and conventionalized cells. 8. Endosperm cells at ten weeks, when the oil is first seen. 9, The nuclei of the flattened endosperm cells of the superficial layer bordering the utriculum. The manner in which the oil appears with reference to the nucleus is evident.

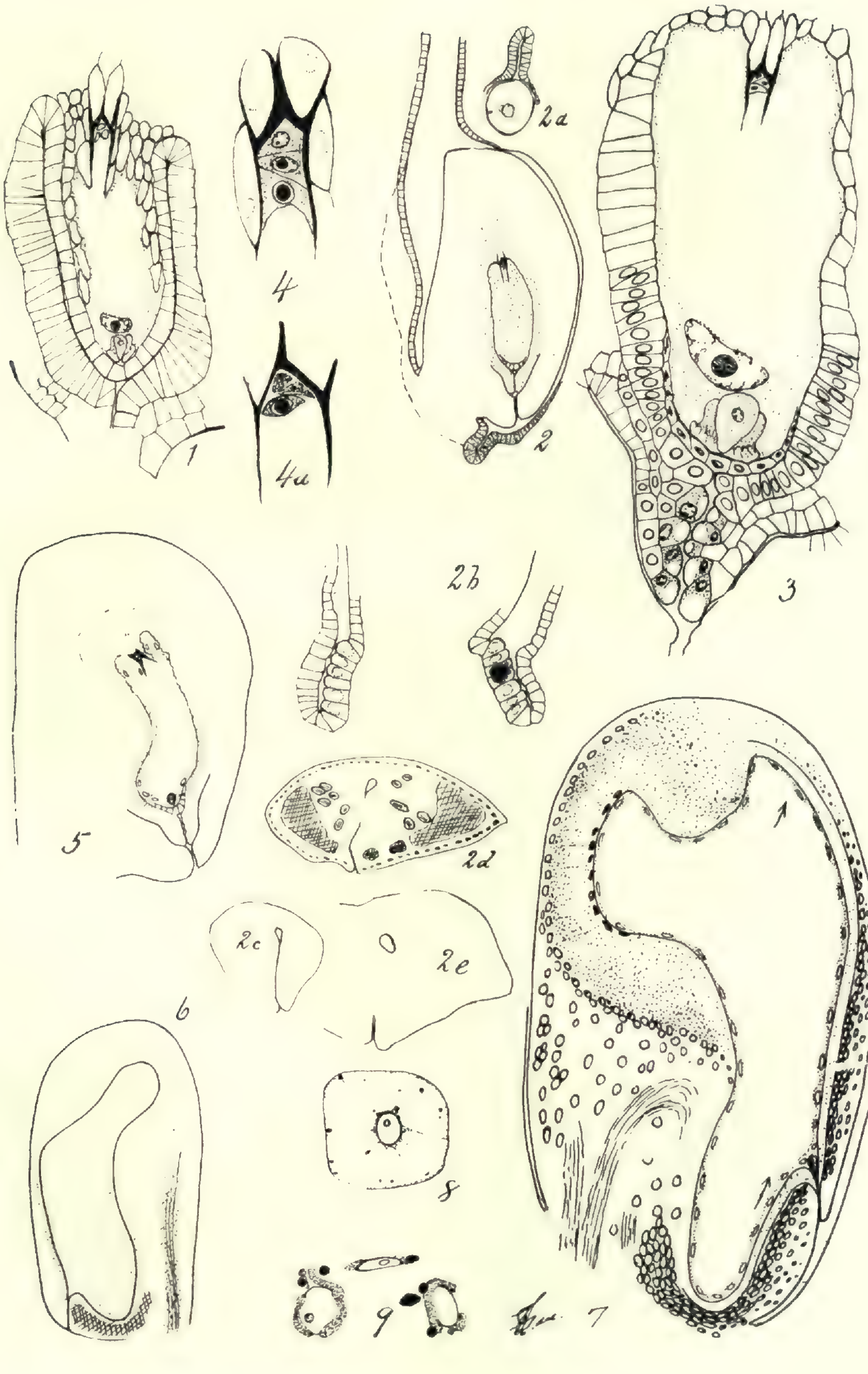
Plate 16.—*Phoenix dactylifera.*—10, (*Cf.* f. 8-9) Ovule, Deglet Noor, 11.5 weeks. Endosperm growing inwardly to obliterate the utriculum. Beginning of oil secretion. 11, Portion of transverse section of carpel and ovule to show the sutural sector in which a diffuse tannin reaction appears. 12, Longitudinal section of mature seed indicating the topography. *Ch.* chalaza. *End.* endosperm. 13, Transverse section through young seed through the chalaza. The chalazal tannin tissue proper is indicated by dots. Tannin is present however, elsewhere in the raphe and integuments. Same ovule as in f. 14. 14, Young seed at the time when the closure of the endosperm is complete. The distribution of oil about the embryo is indicated by the size of the dots. 11 weeks after pollination. 14a, The region about the embryo at about this time, in which no digestive action on the cell walls is observable; *a*, Digestive zone; *b*, Tension zone, where cell divisions occur; *b′*, Thin-walled growing endosperm. 15, Ten

weeks after pollination. Area about the embryo as seen in transverse section soon after the digestion of the cell walls is begun in *a* and *a'*; *a*, Crushed cells empty save of oil drops; *a'*, Uncrushed cells like those in *a*; *b*, Cells in which the protoplasm is being attacked and the oil segregates; *b'*, Thin-walled endosperm between the growing endosperm cells of *b* and those of *c*. 15a, The integuments and edge of endosperm at about this time. As yet no special tannin cells have appeared (*cf.* f. 31); *o. i.* Outer integument. *i. i.* Inner integument. 16, Thirteen weeks after pollination. For full explanation see p.—. 16a, Single endosperm cell in the course of secondary thickening taken from the position in f. 16 indicated by the black square at *x*.

Plate 17.—*Phoenix dactylifera*. 17, About (17 weeks after pollination) and 18, For full explanations of these figures see p. 142. The tannin in the embryo at the age of figure 18 is shown by the dotting, which is intended to indicate the areas of denser reaction only. About 18–19 weeks after pollination. 18a, Camera lucida drawing to show the distribution of oil globules in zones *a* and *a'* of figure 18, in which the same is shown diagrammatically. The irregular particles are protein. 19, Nearly mature embryo; *a*, Crushed cells (*a*, *a'*, f. 20); *b'*, Partially thickened cells (*b'*, in f. 20); *c*, Definitive endosperm. 20, Detail of f. 19 through the endosperm next the embryo; *a*, Crushed cells with minute oil droplets; *a'*, Partially crushed cells with larger oil droplets; *b'*, Partially thickened cells with transverse secondary walls, with large drops and the remains of the protoplasm. Definitive endosperm to the right of *b'*. Deglet Noor. 21, Cells of definitive endosperm which have arisen by secondary division of elongated cells which have resisted digestion. 22, The same as 20 but in front of the cotyledon, showing the final divisions of the endosperm cells and the digestion of their contents. 23, Detail to show the position of the secondary division walls in the endosperm which resists digestion. *a*. Crushed cells. *b''* partly thickened cells with their walls at right angles to the axes of the endosperm cells from which they arose. 24, Haustorium and adjacent endosperm of seedling with extraseminal portion 2 cm. long; *a*, Crushed walls, blue with iodine; *b*, Crushed walls, not blue with iodine; *c*, Cytatic digestion advanced, oil agglomerated; *d*, Cytatic digestion beginning, oil agglomerating, protein digesting; *e*, unchanged endosperm. 25, A younger stage than 24, showing the embryonic layer blue-reacting with iodine still intact. Cytatic digestion has begun in front of the cotyledon. Dots in the embryo indicate tannin; cross-hatching, starch.

Plate 18.—*Phoenix dactylifera*. 26, Embryo and adjacent endosperm showing the distribution of tannin. 11 weeks after pollination. 26a, Transverse section of the same seed as 26, showing the areas in which the secondary thickening of the endosperm cells has begun. Here tannin is abundant, as shown by the dots. 27–29, Successively older stages showing the spread of the endosperm tissue

with thickened walls and large tannin content: 27 shows the tannin in and near the embryo; 28 shows the tannin in the embryo; 27a. Large tannin content of the endosperm cells of the chalazal cul-de-sac where the secondary thickening of the cells has gone on as in the main mass of endosperm. 30. Transverse section of seed near maturity in the endosperm of which tannin still is present in certain sectors. 31, The integuments in their definitive condition. All the cross-hatched cells contain tannin. 32, Exocarp. Diagrammatic representation of the various tissues; *e*, Epidermis; *s*, Stone cells; *p*, Parenchyma, of which a few cells are drawn here and there to show their relative sizes; *id.* tannin idioplasts. 33, Outer exocarp to show the tannin cells in the hypodermal layer, and the tannin globules in the parenchyma adjacent to the stone cells, st. (Rhars). 34, Endocarp in transverse section, *t*, elongated tannin element.



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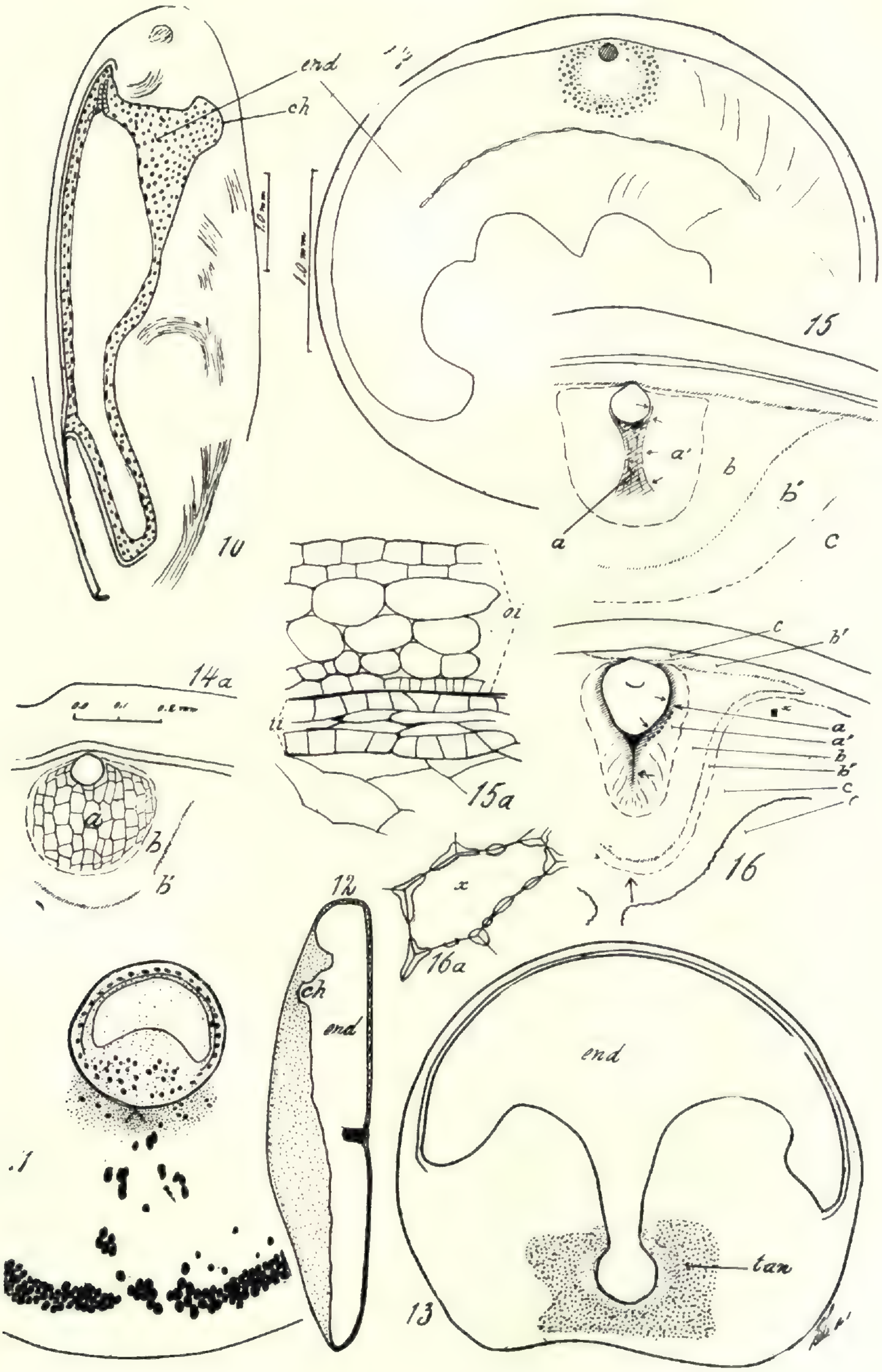

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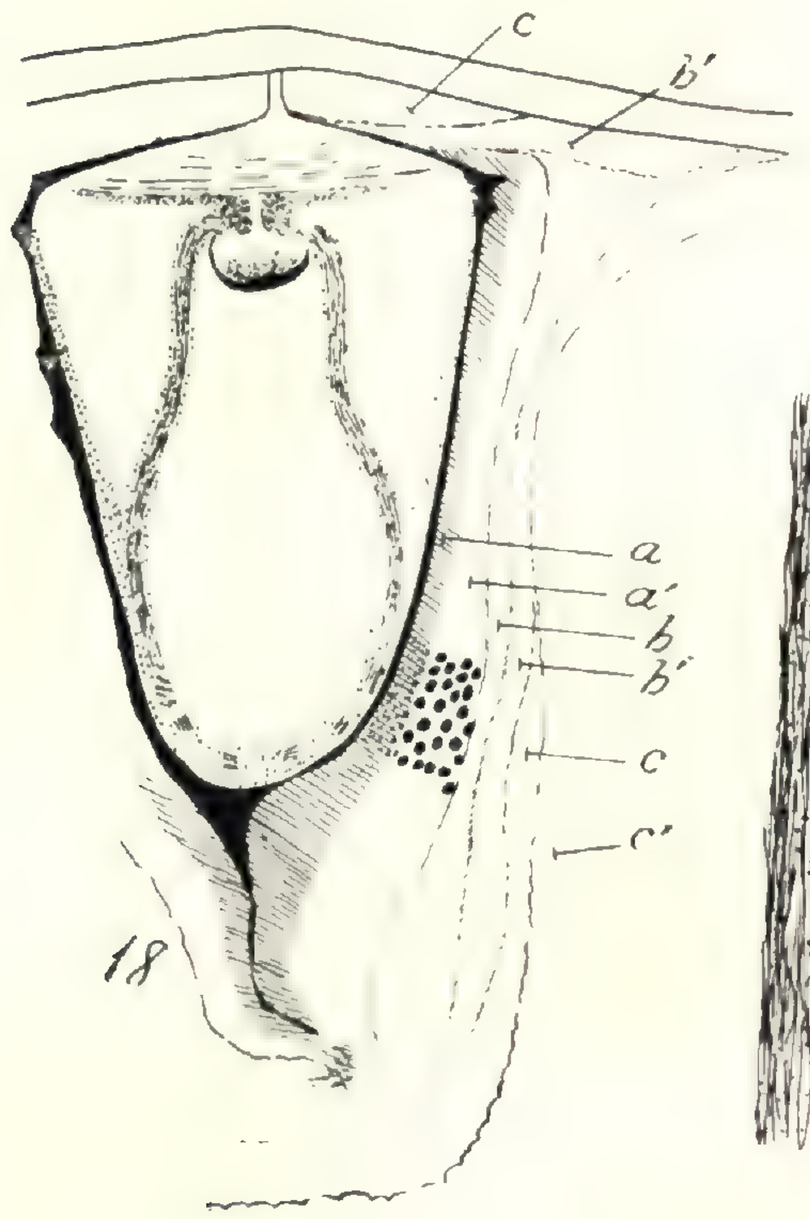

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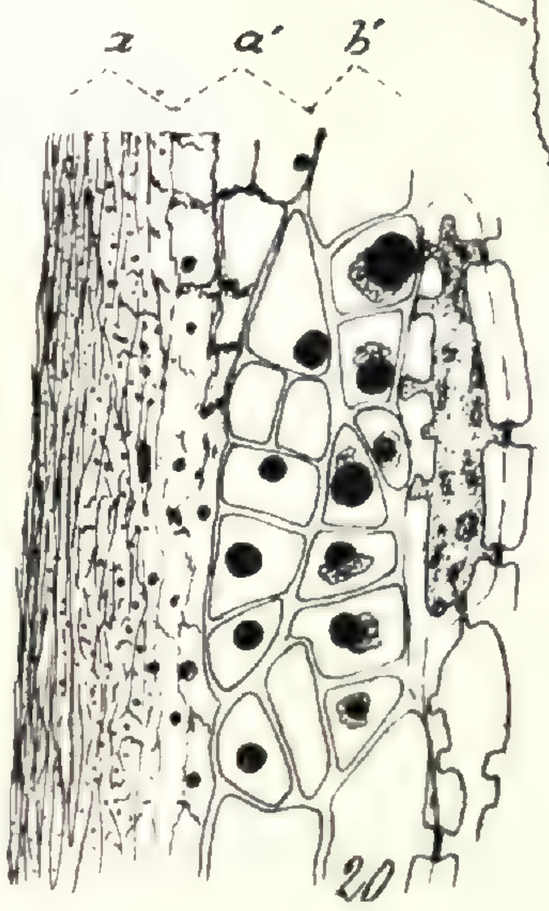

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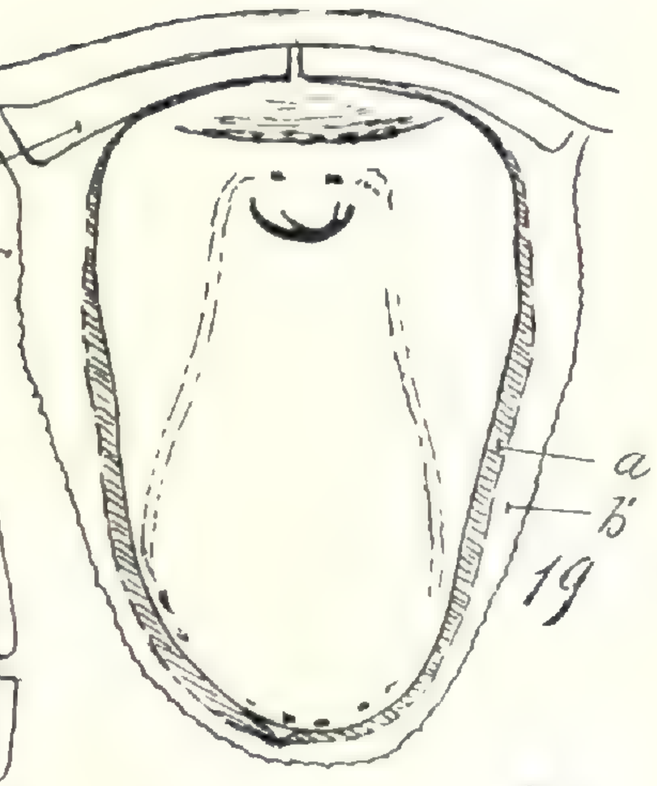

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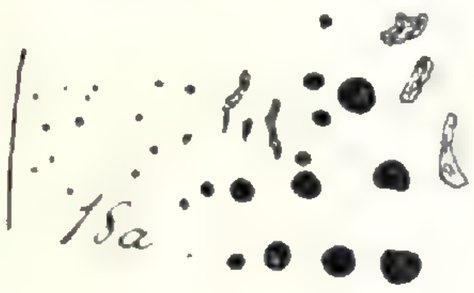

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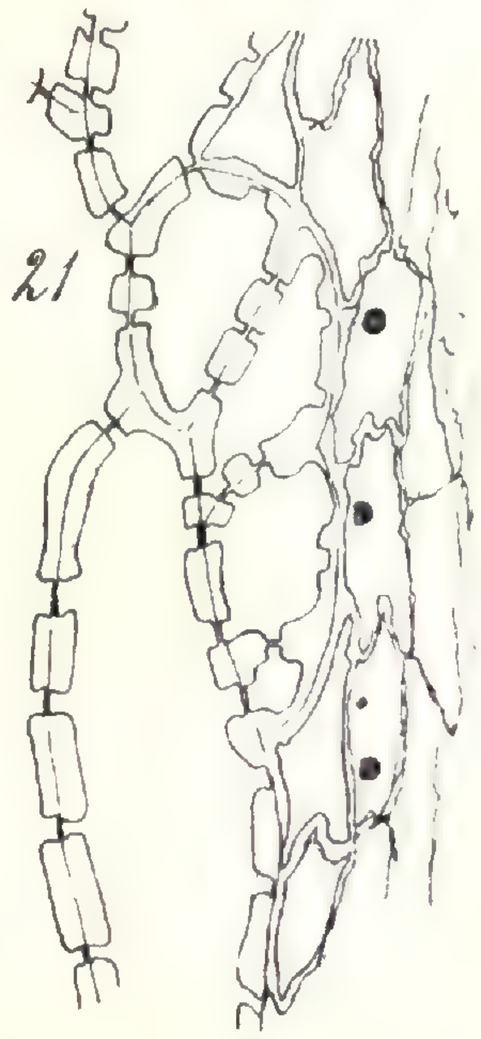

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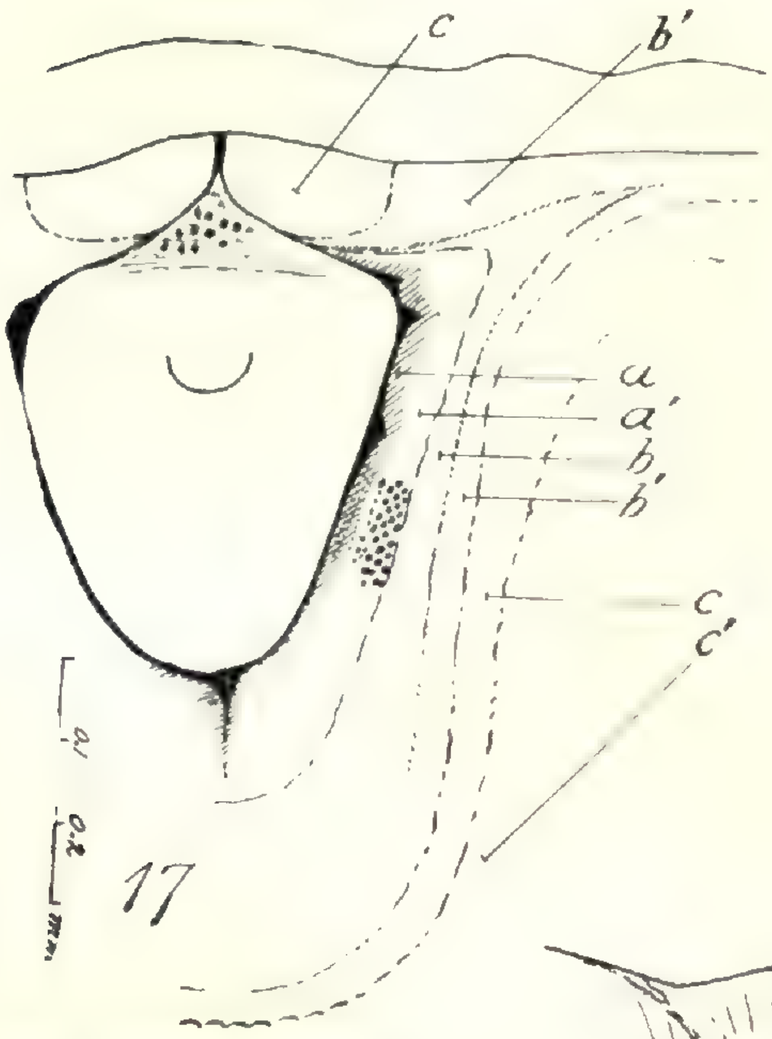

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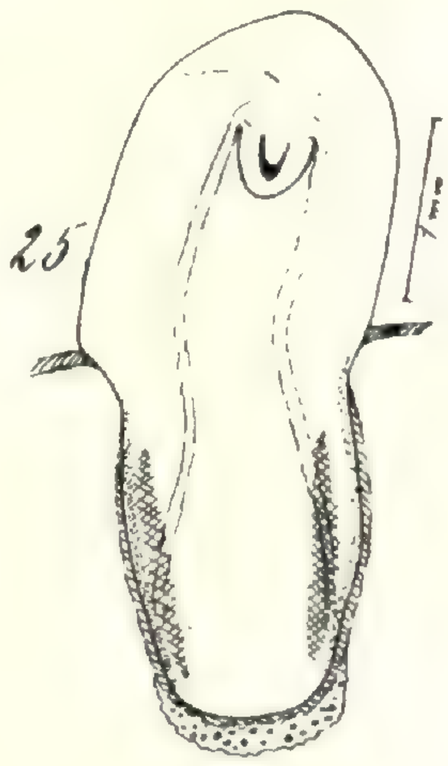

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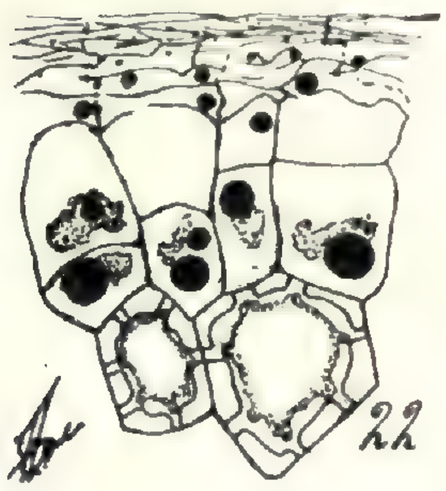

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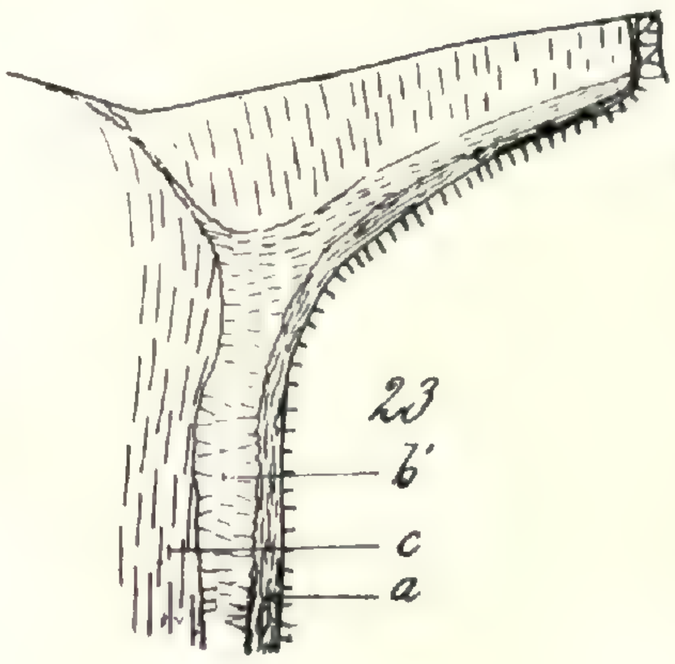

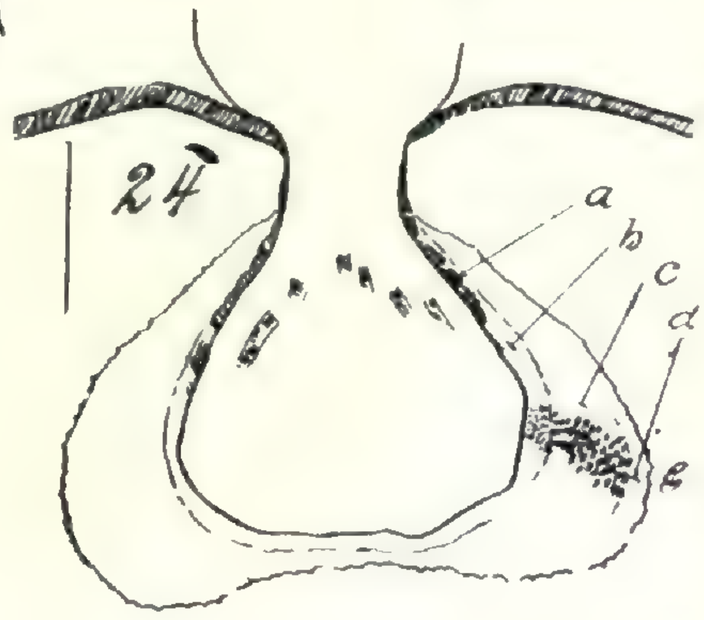

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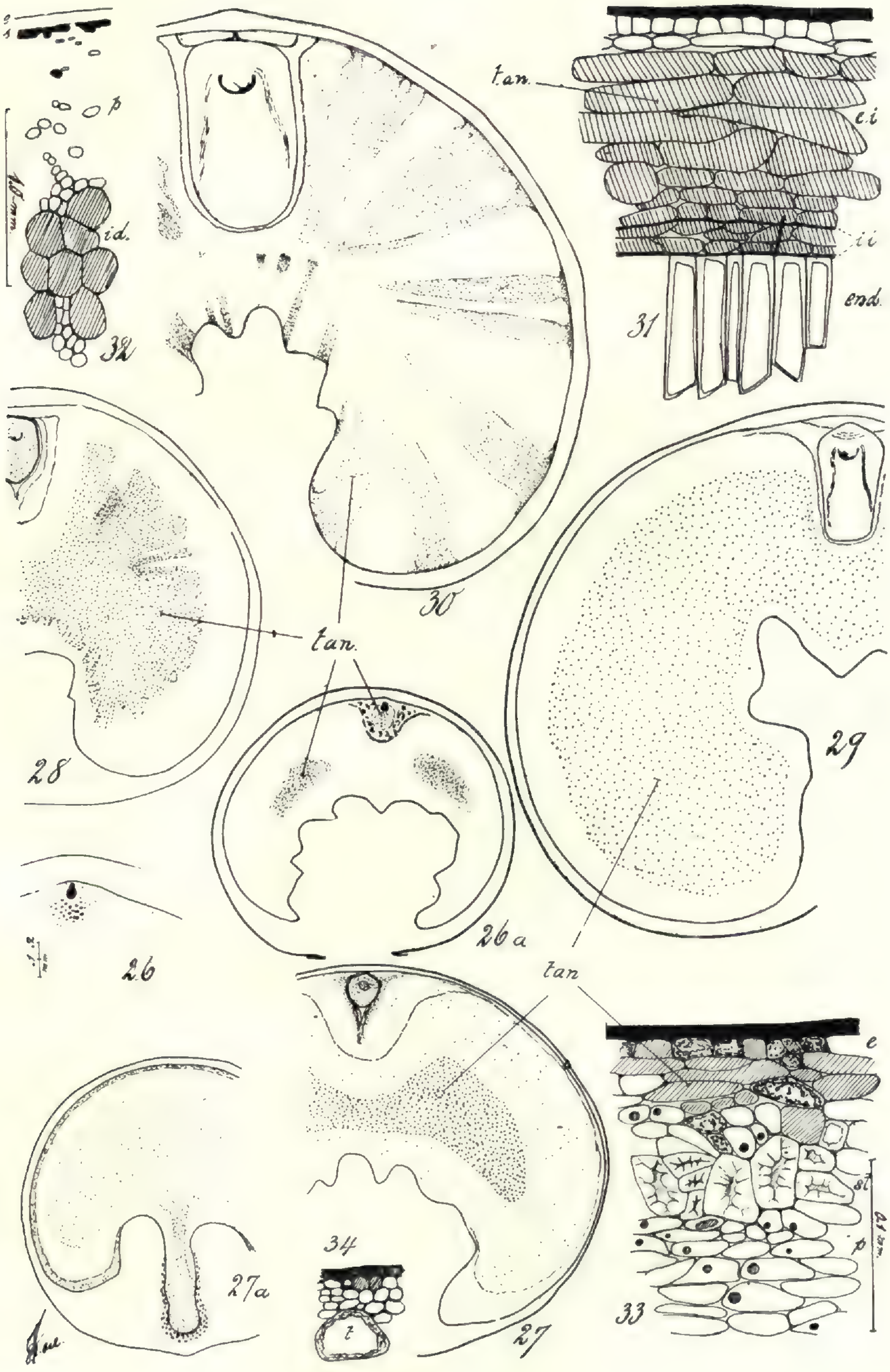


PHOENIX DACTYLIFERA.

ILLUSTRATED STUDIES IN THE GENUS OPUNTIA—III.

BY DAVID GRIFFITHS.

Studies in field and cultivated plantations during the past five years have brought together sufficient data to warrant the addition of the following species to the genus *Opuntia.*

Opuntia alta sp. nov.

A strictly arborescent species with distinct, short, cylindrical trunk, 3 to 3½ dm. in diameter and huge, spreading branches 1½ to 2 dm. in diameter, early becoming bare, brown-gray and scaly-cracked, 2½ to 3½ meters high in large specimens, mostly lower than this and often with a spread of 3 meters, mostly about 18 dm. high; joints sub-circular, ovate to obovate, quite uniform in size, about 18 by 25 cm. or possibly more often 17 by 21, blue-green, thin, with surface only slightly raised at areoles, turning gray-green and scaly-cracked in age; areoles obovate, about 3 to 5 mm. long and 3 cm. apart, slightly raised, closer on edges where they are also larger, rendering a somewhat congested appearance to spines and spicules, enlarging with age to sub-circular and often 1 cm. in diameter, tawny when young, becoming dirty black in age; spicules yellow, abundant, 8 mm. long, mostly scattered throughout the entire areole but more numerous above, increasing with age and filling and crowding the entire areole, the tissues of which proliferate slightly into a raised hemispherical structure, the outer spicules becoming dirty yellow and the central newer ones a brighter color; spines yellow, typically, one 15 to 20 mm. long, erect, and one 10 to 12 mm. long, and sloping down on sides of joints and two or even 3 long ones on edges, not increasing with age to any appreciable degree, at about 5 years of age and older the trunks becoming comparatively bare of spines but covered with the scattered, formidable, bunches of spicules only, slightly flattened and the largest ones faintly annular; flowers yellow, with broadly-rounded, wavy-margined, obovate petals, with abrupt cuspidate point, filaments yellow, greenish at very base, style white, stigma yellowish tinged, 10 to 12-parted; ovary broadly obovate to conical, about 2 by 3 cm. having small sub-circular areoles bearing spreading, unequal, yellow, fugacious spicules about 5 mm. long.

This species is distinctly arborescent in habit, one of the tallest and largest of our United States forms. It is very different indeed from *Opuntia cacanapa,* although one or

two of its characters seem to indicate a relationship. The type is yellow flowered, but there are forms which have flowers greenish-yellow, even lighter in color than the flowers of *Opuntia leptocaulis.* Owing to a constant tendency to segregation of species, it is considered preferable to recognize the yellow flowered form as typical, but I have little question but that the two forms must be considered specifically the same. The greenish-yellow flowers remain the same color throughout the day, but in the typical form where the flowers are lemon yellow they turn to orange in the afternoon, and when dried or closed upon the plant are distinctly reddish tinged, which is true of the type specimen. Corresponding changes in color take place in a large proportion of the species of the flat-jointed opuntias.

The description is a compilation of a description and two sets of notes made in the type locality when specimens were collected. The type is No. 9914 D. G., collected March 13, 1910, near Brownsville, Texas, the flowers being collected from the same plant April 20, 1910.—Plates 19 and 20, upper figure.

Opuntia xanthoglochia sp. nov.

An erect to ascending, spreading, tuberous rooted species with radiating arms frequently resting on their edges on the ground, seldom over 3 dm. high, but often 8 or 9 dm. in diameter; joints usually widest near middle, but sometimes obovate, mostly more or less pointed above and below, about 10 by 15 cm., dark green, glaucous when young, wrinkled and decidedly raised-tubercular at the areoles; areoles about 4 mm. long, obovate, about 2 cm. apart, enlarging and becoming sub-circular in age, sometimes 1 cm. in diameter; leaves subulate, cuspidate, slightly flattened; spicules light brown when young, but soon turning light yellow, conspicuous and formidable, in compact, 4 to 5 mm. long tufts in upper portion of areole, increasing with age, the new ones coming from center of areole and longer from year to year, the annular growths being in concentric circles, but brown color only appears on young joints, the change in color beginning to take place early in May; spines delicate, flattened, often twisted, usually 1 or 2, the longest about 2 cm., erect, or when 2 or 3, one sloping downward; flowers lemon yellow, brownish red within, lax, 7 cm. in diameter, turning very light orange to pinkish, filaments greenish, style white, stigma white or very light yellowish, 5 to 6 parted; ovary long, obovate or obconical, somewhat tubercular with raised areoles which are small and sub-circular, about 12 mm. by 6 cm.

The plant belongs to the *Opuntia macrorrhiza* group, but, as will be noticed from the description of the plant body, it is very different from that or any other of the related species. The flowers are exceedingly variable in character. At times they are pure lemon yellow throughout with no indication of red at base of the petals. At other times the lower one-third may be red and all degrees of variations between these two extremes may be found. Sometimes the red is confined to the mid-rib of the petal, being uniformly distributed throughout its length.

The description is drawn from a cultivated plant grown from a single joint cutting set July 11, 1908. It was collected on that date near Milano, Texas. The description of the flowers and the notes on the flowers were made in the type locality from the type plants May 15, 1910. The type specimen is No. 9355 D. G., prepared May, 1910, from cultivated specimens bearing the same number.—Plate 20, lower figure.

Opuntia Gomei sp. nov.

Plant ascending with main branches commonly on edge and secondary ones erect from them, mostly about 1 meter or less high and often 3 or more meters in diameter, the old centers containing much dead material when plants are large; joints sub-circular to ovate, rarely blue-green, varying to sometimes slightly yellowish green, 30 to 40 cm. in diameter, or often in last year's joints only 20 cm. in diameter and then almost invariably sub-circular, more or less raised at areoles and larger joints always more or less wavy; areoles obovate, 6 to 10 mm. in diameter, bright brown when young, changing to dirty brown and finally to gray-black, mostly raised and surrounded by an irregular, dry, brown-gray, slightly cracked rim or area, varying from 2½ to 5 cm. apart; spicules yellow, abundant, very prominent, about 1 cm. long, more abundant above, but often filling entire areole; spines yellow, somewhat flattened, often faintly annular, not twisted, 3 to 5 cm. long, erect, divergent, sometimes increasing irregularly with age, 1 to 5 in number, mostly 2 to 4; flowers yellow, stigma large, bright deep green, 9 to 10 parted; fruits reddish purple.

This species belongs to the *O. Lindheimeri* group. It inhabits the lower edges of the slight elevations in the delta of the Rio Grande River and often extends both into the huisache flats and the mesquite areas of higher elevations.

It is, therefore, in all probability, able to thrive upon land containing considerable soluble salts. It is often found growing scatteringly upon land entirely devoid of brush and which periodically overflows. In these areas, however, it frequently gets killed out on account of the overflows which occur at irregular intervals, and again becomes established thinly before another period of overflow occurs.

The description was drawn in the field when the type specimen was collected, notes on the flowers being subsequently secured. The type specimen is No. 9913 D. G., collected near Brownsville, Texas, March 13, 1910. It is named in honor of Mr. William Gome, whose assistance it is a pleasure to acknowledge.—Plates 21 and 22, lower figure.

Opuntia pachona sp. nov.

Plant tall, arborescent, widely branching but not as divergent as *O. streptacantha*, with distinct cylindrical trunk 1 to 1.5 meters long, the whole plant normally 3 to 5 meters high; joints about 20 by 32 cm., obovate, deep dark green, with often a white bloom, similar to *O. streptacantha* in color but on the whole a little lighter; areoles 2½ to 3 cm. apart, ovate to sub-circular; spicules bright dark reddish brown, often formidable, in compact tufts in upper part of areoles, increasing in numbers and length with age, about 2 mm. long; spines white with bonelike tips, flattened or triangular, often slightly twisted, mostly slightly but never tightly recurved, 2 to 5 or 6 in number, mostly 2 to 4 on last year's joints, increasing on old trunks to 6 or 8 and becoming larger and stouter than on young joints, diverging in all directions upon old trunks although upon young joints they are slightly recurved or sloping downward; fruits about 40 to 45 by 50 to 55 mm. bright, glossy, purplish-red when mature, beset with rather large circular areoles containing formidable reddish brown spicules surrounded by the blackened ends of the dark tawny wool.

Opuntia pachona belongs to an important group of prickly pears of the highland of Mexico in which the expressed juice contains a large amount of solids in suspension. It is, therefore, one of the forms used in the manufacture of queso. Although similar in color to *O. streptacantha,* it can hardly be considered as closely related to this species, but should be looked upon as representing the *Opalillo-Lionero* group none of which, so far as I am aware, have satisfactory descriptions.

The description is a compilation from two sets of char-

acterizations, modified by several miscellaneous notes from various localities, one description having been written in the type locality and the other drawn from a three year old seedling. The type specimen is No. 8141 D. G., collected near Zacatecas, Mexico, September 19, 1905.—Plate 22, upper figure.

Opuntia lubrica sp. nov.

A low ascending, spreading species very similar in habit to *O. microdasys*, frequently 4½ dm. high and when well developed 10 dm. or more in diameter; joints sub-circular to obovate, about 15 by 20 cm., or in case of last joints of previous year about 12 by 15 cm., bright, glossy, leaf-green, very evidently papillate but scarcely pubescent under a lens; leaves subulate, cuspidate-pointed, 6 to 9 mm. in length; areoles 15 to 22 mm. apart, 4 to 6 mm in diameter, sub-circular, prominent; spicules prominent, 4 to 5 mm. in length, erect, bushy, in crescentic tufts in upper portion of areoles, becoming much more numerous in age, and at 2 to 4 years completely filling the areole, and, like *O. rufida* and some other species, becoming very abundant and conspicuous by proliferation of areolar tissue into short raised or columnar structures; spines exceedingly variable, sometimes nearly absent, again quite abundant and irregularly distributed, none too many, mostly 1 to 3, becoming more numerous with age and in scattering areoles to as high as 16, mostly about 12 mm. long, but sometimes 2½ cm., yellowish, translucent, bonelike, sometimes darker at base; fruits decidedly acid, light red without with yellowish green rind and red pulp; seed small, thin shelled, about 3 mm. in diameter.

The habit of this species resembles that of *O. microdasys*, but it is a more robust plant with heavier glossy joints smooth to the touch and with color of entirely different character.

The description is a compilation of partial descriptions made in the type locality and notes upon cultivated, nearly mature plants. The type is No. 8439 D. G., collected near Alonzo, Mexico, August 23, 1906, backed up by several specimens put up from cultivated material. The cultivated plants have not yet bloomed, although one of them is now in the third year's growth from a single joint cutting.—Plate 23.

Opuntia nigrita sp. nov.

An erect, open-branching, stout, arborescent plant with distinct cylindrical trunk and spread of branch about like *O. pachona*, com-

monly 3 to 4 or more meters high, in cultivation plants have made a 12-dm. growth in 2 years from single joint cuttings; joints obovate, broadly rounded above, about 17 by 25 cm., papillate-pubescent under a lens, this scarcely detected by touch except in current season's growth, deep dark green, with young growth of course lighter in color; areoles at first brown turning dirty black, broadly oval to sub-circular or even obovate, about 5 mm. in longest diameter, 2 to 2¼ cm. apart; leaves short, subulate, cuspidate, mostly only 3 mm. in length; spicules brown, in hemispherical bunches in upper part of areole, 2 to 3 mm. long at first, but developing anew from lower central portion of spicular area to often 1 cm. in length, this new development continuing for a couple of years; spines white, turning to a dirty gray, not very stout, but resembling those of *O. chaveña*, but longer, numerous, erect, spreading in all directions, 4 to 6 on last year's joints, but becoming very numerous in places on old trunks, even 25 to 30, varying in some areoles from 15 to 25 mm. in length; besides spines and spicules, current year's joints bear two delicate, hairlike, fugacious spines, about 5 mm. long; fruit small, about 3 × 3.5 cm. purplish red, with pulp deeper in color and seeds easily separable.

The species is one of the tree forms of the highland of Mexico of the southern Zacatecas and Aguas Calientes region. It is very different from any other species with which I am familiar, especially in size of fruit. My field notes indicate that when it was collected it was thought to belong to the *O. chaveña* group. Its brownish red spicules, however, make it appear quite different and its fruits are entirely different. It has been seen in cultivation in several localities and apparently native to the vicinity of Aguas Calientes.

The description was drawn in the main from a cultivated specimen in the third year of its growth from a single joint cutting, amended by notes from the type and other localities in Mexico. No flowers of it have been seen. The type specimen is one bearing my collection No. 8138, prepared from a cultivated specimen which was collected under the same number near Aguas Calientes, Mexico, September 16, 1905.—Plate 24.

Opuntia Ellisiana sp. nov.

Plant spreading, ascending, laxly branched, 10 to 14 cm. high and 14 to 20 dm. in diameter, depending upon moisture and fertility conditions; joints light glaucous blue-green, obovate or ovate, about 20

by 24 cm., slightly elevated at areoles when young; areoles at first white, almost cottony, turning gray and finally black, small, 2 or 3 mm. in diameter; leaves long, prominent, circular in section, subulate, cuspidate, soon recurved, 12 to 15 mm. in length; spicules yellow, but never prominent except on fruit where there are only a few in upper areoles, all but absent from joints; spines entirely absent; flowers deep yellow, changing to orange, reddish when closed, some of the outer perianth segments tinged with dull greenish red in bud, about 6 cm. in diameter when fully open, filaments and style white, stigma very light greenish yellow, 7 parted; fruit pyriform, deep reddish purple.

The species is known only in cultivation and only from southern Texas. It is rather common in gardens at Corpus Christi and Brownsville, especially. It was received first through Mr. James Anderson, Jr., and Professor J. Coswell Ellis, from Corpus Christi, and afterwards collected there by myself. It is only remotely related to any other spineless opuntia described. It is about as smooth as any species, much more hardy than the *O. ficus-indica* group, and is said never to be injured by cold weather at Corpus Christi and is apparently hardy at San Antonio.

The description is a compilation of two sets of notes taken upon a cultivated plant. The type is a specimen bearing No. 8626 D. G., prepared from a cultivated plant, the cuttings for which were secured by myself in Mexican gardens at Corpus Christi, Texas, on 1907.—Plate 25.

Opuntia Wootonii sp. nov.

A very open spreading, ascending species, about 6 dm. high (3 years old) and 1½ to 2 meters in spread of branch, the main limbs horizontal, ascending or resting on edge on ground, the secondary ones erect from them; joints widest near the middle, pointed at either end, about 18 by 32 cm., glaucous, light blue-green becoming more yellow in age; areoles broadly oval, about 5 to 7 mm. in length and 3½ to 4 cm. apart, increasing in size with age, at first tawny and then gray, leaves large, slightly flattened, erect, but recurved at tip in age, subulate, cuspidate-pointed, 15 to 20 mm. long; spicules long, formidable, in an unequal, hemispherical tuft in upper portion of areole, often 1½ cm. long above and on edges of joints, increasing with age and often filling the entire areole; spines very long, formidable, erect-spreading, flattened, twisted, faintly annular, the longest often 9 cm. in length and shortest about 1 cm., but the long ones greatly predominating and more commonly about 7 cm., 4 to 6 on last year's joints and increasing

on old trunks to 8 or 10, the longest ones sometimes 11 cm. long, tips bonelike, changing abruptly to white or often yellowish or even translucent which in turn fades into various degrees of reddish brown or even nearly black at base; flowers yellow to deep orange-red; fruit reddish-purple.

This species has been cultivated several years by Professor E. O. Wooton in whose honor it is named. It is one of the most striking of our native opuntias and is easily recognized by its large joints, pointed at both ends like *O. occidentalis,* and exceedingly formidable, showy spines which resemble those of *Opuntia tricolor* more closely than any other species with which I am familiar.

The description given above is taken from a cultivated plant in the third year of its development, supplemented by notes upon flowers and fruits grown at Messilla Park, N. M., by Professor Wooton, who collected the species originally in the Organ Mountains of New Mexico. The type bears my collection No. 9171 which was prepared May 4, 1910, from a cultivated plant the cutting for which was secured in April, 1908, from Professor Wooton's collection. The species was originally collected under Professor Wooton's No. 3030. The plants from which the above description was drawn bore but one flower bud this year, the third season from planting.—Plate 26, upper figure, and Plate 27.

Opuntia atrispina sp. nov.

Plant 7 to 10 dm. high and 12 to 15 dm. in diameter or often smaller, the main branches spreading with edges on the ground or ascending, the secondary branches mostly erect; joints obovate, rounded above to sub-circular, mostly slightly raised at areoles, about 11 by 15 cm. and 1 cm. thick, yellowish green; areoles obovate, or on young joints mostly elongated and raised below, about 5 mm. long and 25 mm. apart; spicules yellow, prominent, unequal, occupying a large triangular area in upper part of the areole, but finally scattered throughout its entire area but more numerous above, 6 to 12 mm. long, increasing with age; spines jet black to reddish brown at base with yellow tips, the transition between the two colors being quite abrupt, but the proportion of the two colors varying tremendously in different individuals, mostly one large, sub-erect one, 25 mm. long and one smaller, recurved one about 13 mm. long immediately below it and 2 shorter beside and a little below the latter about 6 mm. long; scattered among the spines are a few fugacious, reddish brown spic-

ules with yellow tips, all finally fading to a dull dirty gray or brown; flowers yellow changing to orange, about 4 or 5 cm. in diameter when fully opened, greenish within with filaments yellowish above and greenish below, style white, stigma yellowish, small, 7 parted; fruit small, pyriform, slightly to quite deep pitted above, reddish purple without and greenish yellow within, rind quite succulent and pulp small in amount having a slight aroma when first cut, covered with small, sub-circular areoles not over 1 mm. in diameter and 5 or 6 mm. apart.

The species is more closely related to *O. phaeacantha* than any other species, but differs decidedly in many characteristics as indicated above. Well matured plants are quite different in habit. Although always greenish yellow within, the fruits differ decidedly in size.

The description was drawn in the type locality and has been amended by subsequent notes secured near Devil's River, Texas. The type is No. 9411 D. G., collected near Devil's River, Texas, July 20, 1908.—Plate 26, lower figure.

Opuntia Sinclairii sp. nov.

An erect or ascending, open branching species making a shrub 12 dm. high and 1½ to 2 meters in diameter; joints broadly obovate and broadly rounded above, commonly 20 by 22 cm., although often larger and smaller, blue-green, mostly somewhat glaucous, especially when young; areoles sub-circular to obovate, 5 to 6 mm. in length, at first brown, becoming dirty black; leaves 6 to 8 mm. long, sub-circular in section, subulate, cuspidate-pointed, mostly recurved; spicules reddish brown, numerous, scattered, unequal, 5 to 6 mm. long, the tips often fading to yellow and even dirty grayish; spines yellowish, bonelike to chalky white with light brown bases, mostly 3 or 4, often 2 to 5, erect, divergent, increasing with age to often about 8, the longest 4 to 4½ cm. long, flattened, usually not twisted, faintly when at all annular; flowers yellow, fading to orange, filaments yellow, style white, stigma bright green, 8 to 9 parted; fruit reddish purple throughout; seed small.

This species is rare in the type locality and is rather closely related to *Opuntia Lindheimeri,* from which it differs in having reddish-brown spicules and spines colored at base, these characters being constant and distinct. It has been in cultivation with us now for the past 6 years and usually produces flower and fruit in abundance the third year from single joint cuttings.

The description is a compilation of several drawn from a number of cultivated plants, together with subsequent notes upon the flowers. It is named in honor of Mr. Wm. Sinclair to whom I am greatly indebted for assistance. The type is No. 9003 D. G., prepared from a cultivated specimen May 2, 1910. The original cuttings were secured near San Antonio, Texas.—Plate 28.

EXPLANATION OF PLATES.

Plate 19.—*Opuntia alta*, from type plant.

Plate 20.—Above, *Opuntia alta*, type plant. Below, *O. xanthoglochia*, from cultivated plant.

Plate 21.—*Opuntia Gomei*, from type plant.

Plate 22.—Above, *Opuntia pachona*, showing a diseased spot, from a cultivated seedling. Below, *O. Gomei*, type plant.

Plate 23.—*Opuntia lubrica*, from cultivated plant grown from cutting.

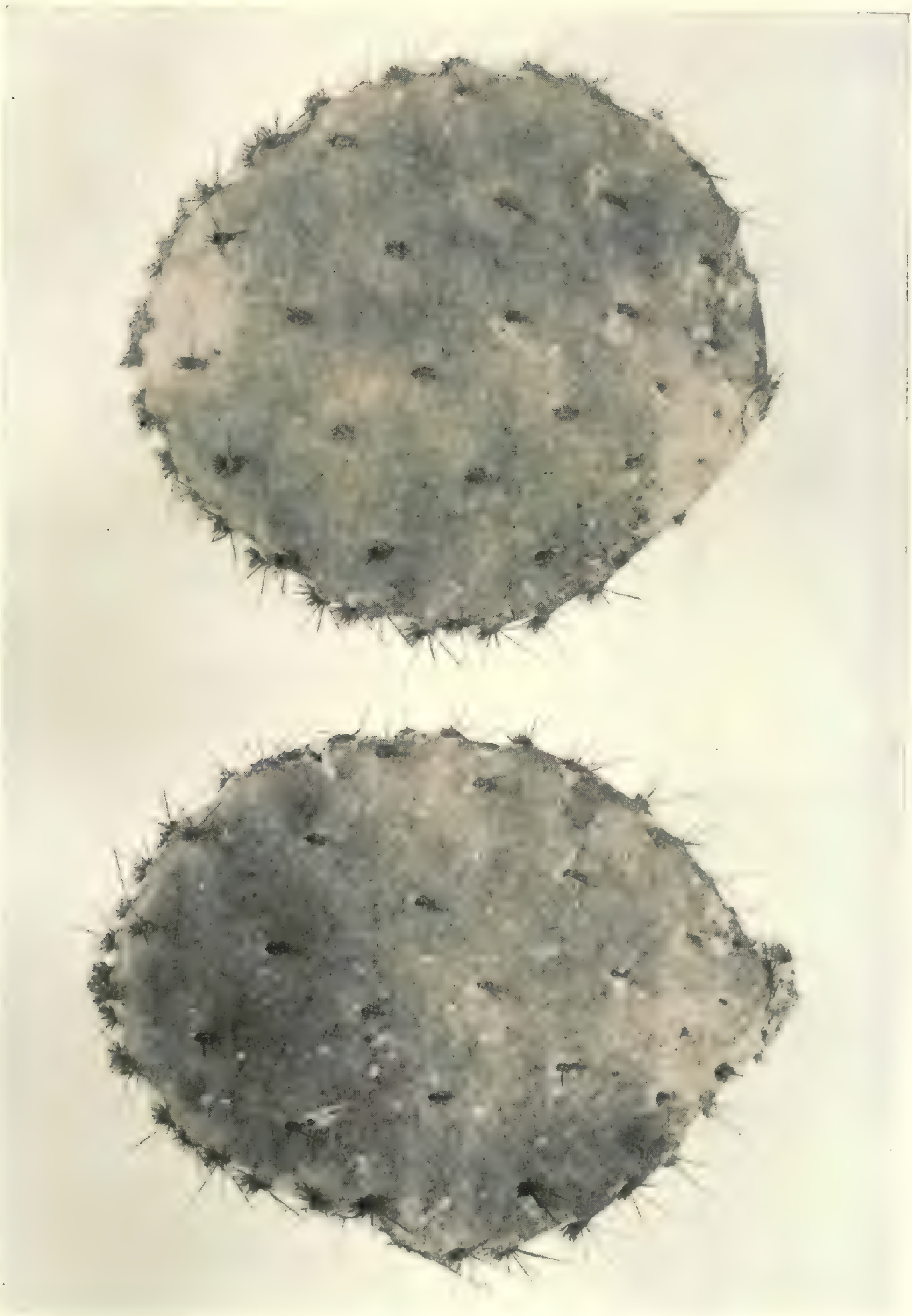
Plate 24.—*Opuntia nigrita*, from a nearly mature plant cultivated from a cutting.

Plate 25.—*Opuntia Ellisii*, from a cultivated plant grown from a cutting secured in cultivation at Corpus Christi, Texas.

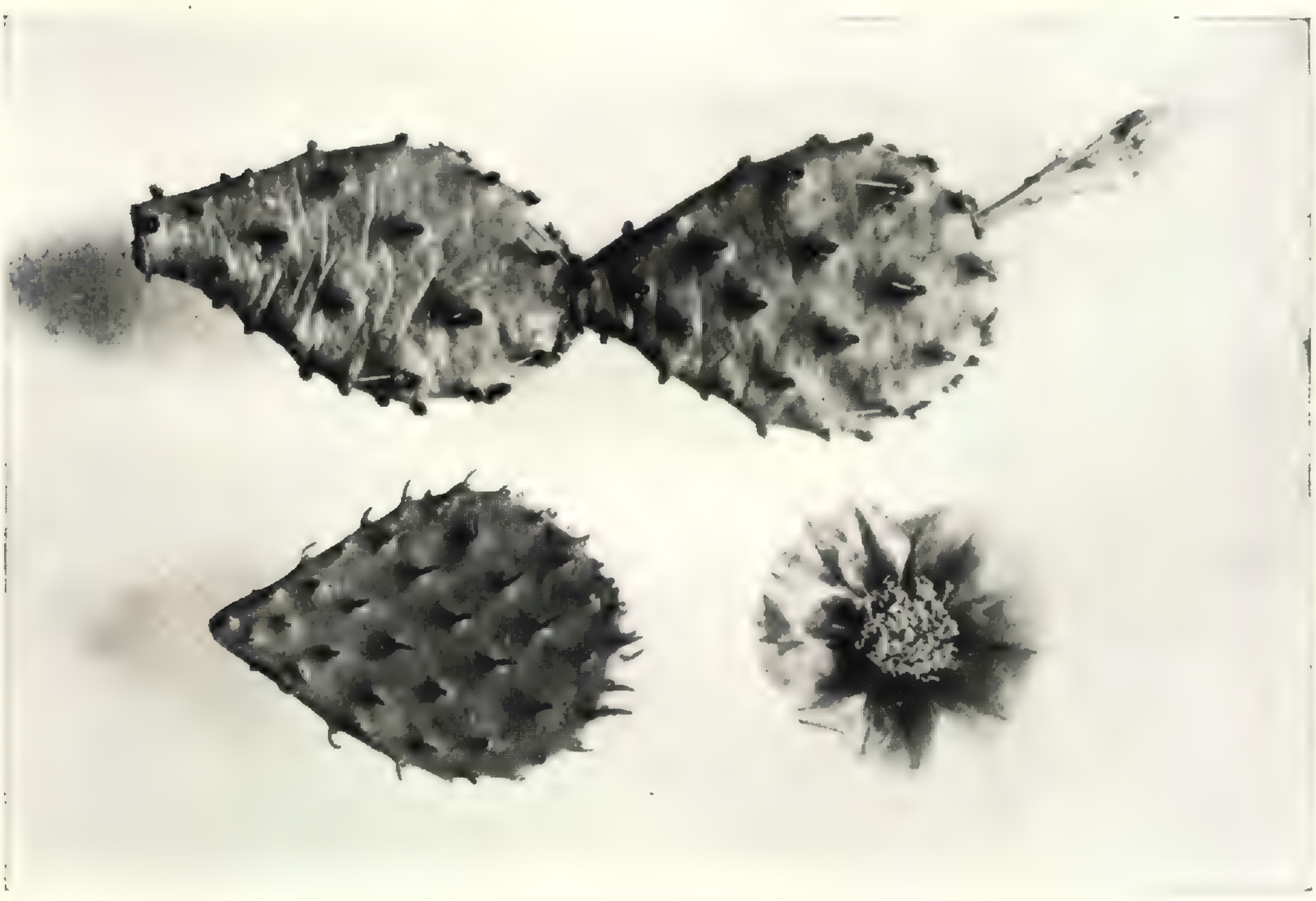
Plate 26.—Above, *Opuntia Wootonii*, in third year's growth from a cutting from Professor Wooton's plantation. Below, *O. atrispina*, from Devil's River, Texas. A small plant.

Plate 27.—*Opuntia Wootonii*. See upper figure in plate 26.

Plate 28.—*Opuntia Sinclairii*, from a plant cultivated in the type locality.

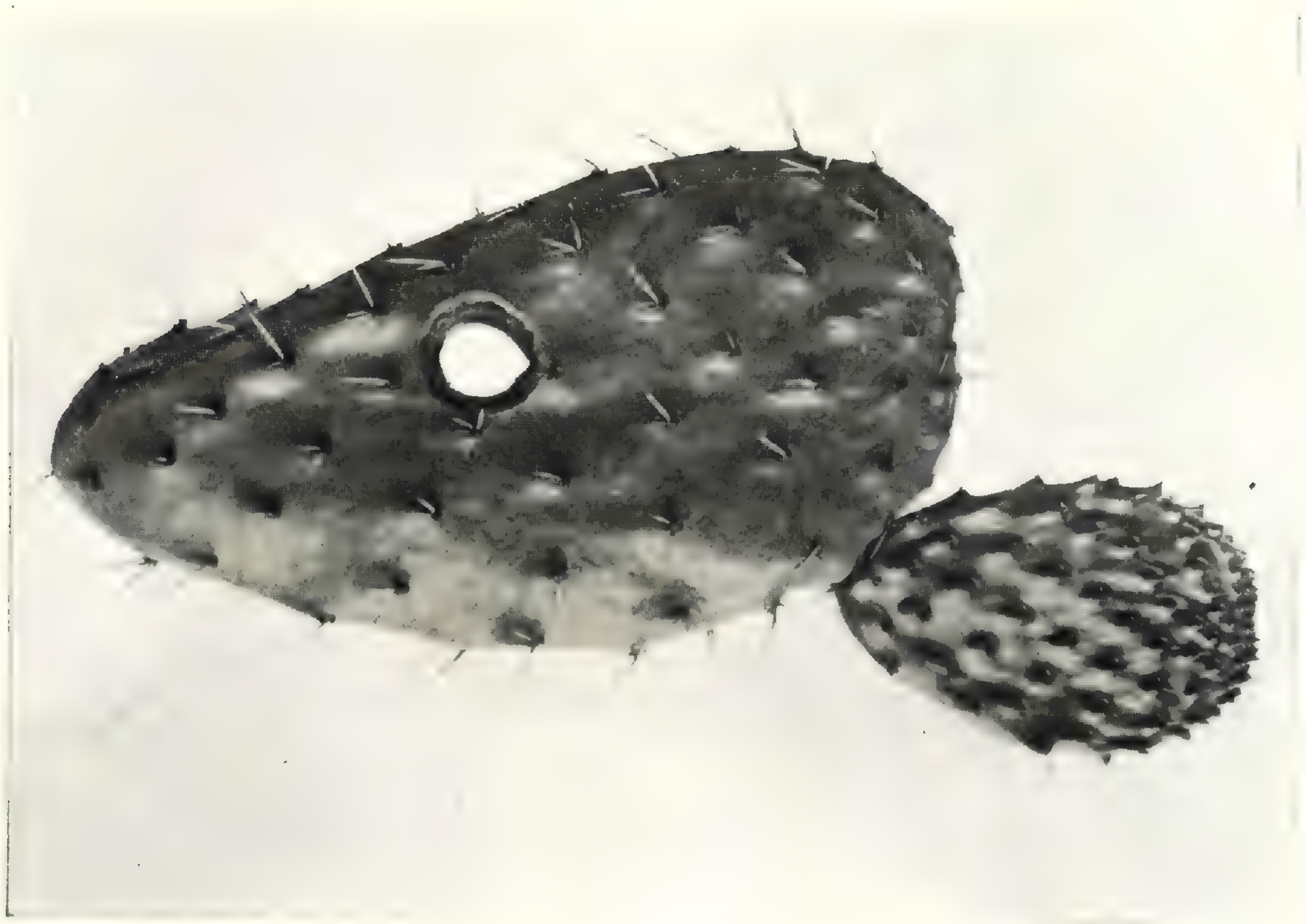


OPUNTIA ALTA.



OPUNTIA ALTA AND O. XANTHOGLOCHIA.

OPUNTIA GOMEI.



OPUNTIA PACHONA AND O. GOMEI.

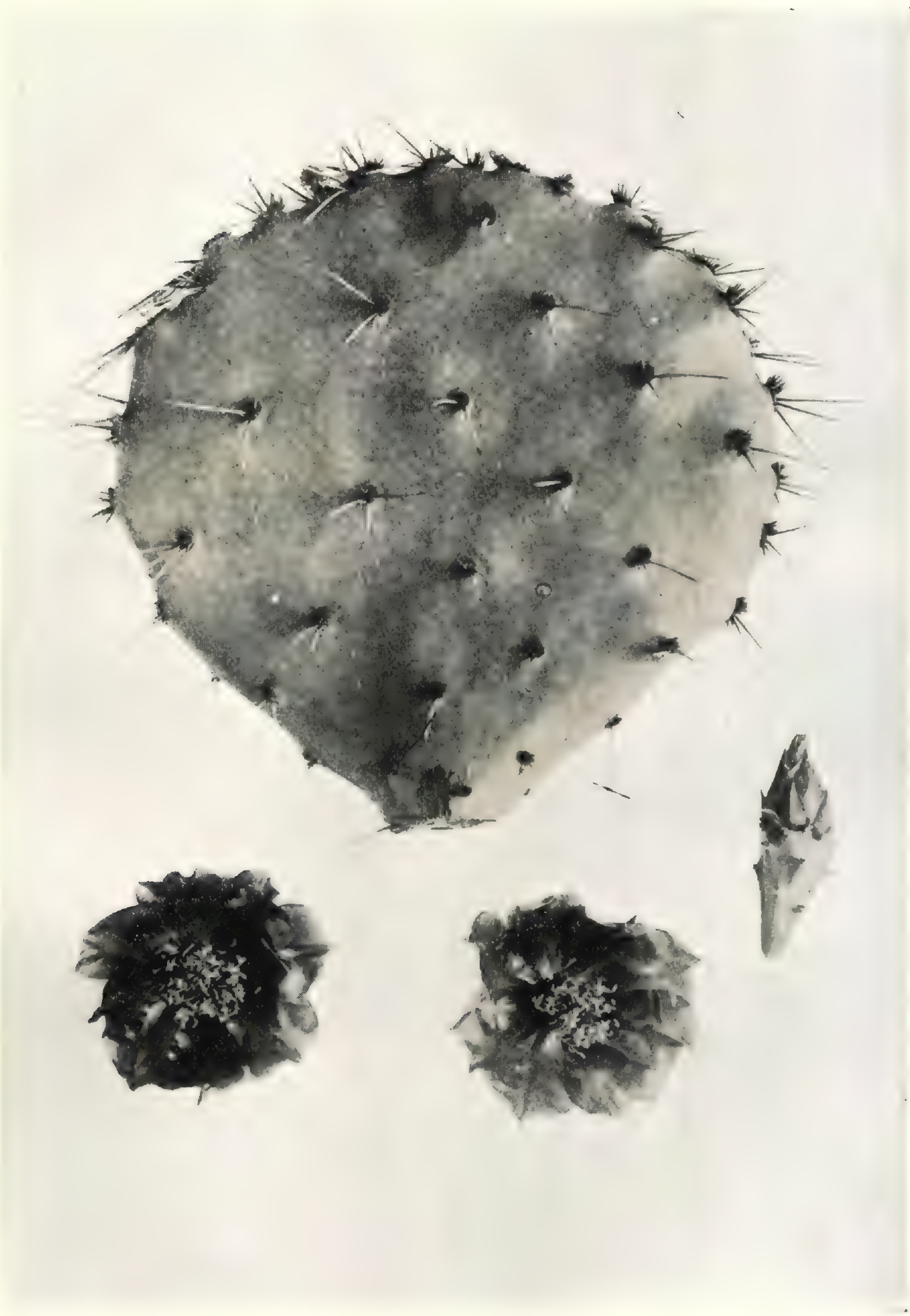
OPUNTIA LUBRICA.

OPUNTIA NIGRITA.

OPUNTIA ELLISIANA.

OPUNTIA WOOTONII AND O. ATRISPINA.

OPUNTIA WOOTONII.



OPUNTIA SINCLAIRII.

ABNORMALITIES IN OENOTHERA.

BY R. R. GATES.

In connection with my *Oenothera* cultures, particularly among plants grown during the past two seasons at the Missouri Botanical Garden, I have had occasion to observe several interesting "abnormalities" of structure. These include virescence or frondescence and polymery of the flowers, tricotyly and variegation of leaves. I have thought it worth while to devote a short paper to a description of some of these cases which have an evident bearing on problems of variation and inheritance.

VIRESCENCE.—In my experimental garden of 1909 four plants exhibited virescence of the flowers. These were all descendants in the second generation from plants which were derived from the English coast near Liverpool, the first generation having been grown at Woods Hole, except in the case of one (No. 47), which was grown in the tropical greenhouse at the University of Chicago. These four plants were therefore all from cultures of closely related forms, and in some of their characters were intermediates between *O. grandiflora* and *O. Lamarckiana.* The summer temperature at St. Louis in 1909 ranged exceptionally high, reading 100° F. in the shade in one instance. The change in climate which the plants experienced was therefore very considerable, and one of the cultures had been subjected to such high temperatures for two successive seasons. This may perhaps have had something to do with the appearance of these cases of virescence, the alteration in the conditions acting as a stimulus to the production of the abnormality. That the tendency to produce virescent individuals is inherited, is shown by the reappearance of virescent plants in one race in successive generations, and their failure to appear in many other races, *e. g., O. Lamarckiana* and its mutants.

Two of the cases of virescence in 1909 occurred in a race which I have called *O. multiflora,* the description of which will be published at another time. This race is descended from a single individual grown at Woods Hole in 1908. A total of 376 first-generation offspring of this individual have been grown in the two following years, and also (in 1910) 50 plants of the second generation from the self-pollination of one individual of the first generation. The plants of the F_1 included a total of 15 virescent individuals, or very nearly 4%. The 50 plants of the F_2 contained one showing virescence. In a culture of 36 plants from seeds received from the Botanical Garden at Karlsruhe under the name *O. chilensis,* which proved to contain two very distinct types, one plant was virescent. This abnormality has not appeared in any others of the many races of which I have grown cultures.

All the plants showing virescence were affected in exactly the same way, although in some the early flowers were normal and produced fruits, only the later flowers showing the peculiarity. I have not compared the offspring from such capsules with those of normal plants, though if this were done it might be found that the virescent tendency was inherited more strongly in the former case. In one plant a side shoot produced flowers which were quite normal while the main stem produced only flowers of the virescent type.

One plant of *O. multiflora,* in which all the flowers but the earliest were virescent, is illustrated in plate 29. The peculiarities of structure exhibited by these flowers may now be described. Plate 30, f. 1, shows a group of the flowers, natural size. The sepals are green inside and outside, large and bag-like and more or less crinkled or curled. They are tapering at the end, terminating in long, slender sepal tips. Perhaps frondescence or phyllody would be a more suitable term than virescence to apply to this condition, for the sepals have become quite leaf-like. Plate 30, f. 2, shows several flowers opened and photographed to show the other organs of the flower. The petals retain a greenish yellow color, but are in all cases very small (usually about half an inch in length, though sometimes larger) and blunt at the tip. The

anthers are small, with very short filaments, empty and sterile. The style is frequently markedly pubescent almost to the top. It tapers strongly and gradually to the top which is very slender, and the stigma lobes are reduced to four delicate prongs.

A remarkable peculiarity of all these flowers is the complete, or almost complete, suppression of the hypanthium. I have remarked elsewhere (Gates, 1910, footnote, p. 208) that the attacks of a certain insect also lead to suppression of this organ. Its wide variability, which Shull (1907) has proved statistically, and its suppression under various abnormal conditions, as I have shown, are probably significant facts, related to its recent phylogenetic development as suggested by MacDougal. A marked feature of this type of virescence is that the flowers do not drop off but remain permanently attached to the stem. In many cases an elongation occurs below the ovary. This is more slender than the ovary and is hard and woody, tough, and strongly attached to the stem. In the meantime (see plate 30, f. 2, flowers to the left) leaves grow out from the interior of the flower and in this way the flower becomes transformed into a short side branch. The ovary in the meantime almost completely disappears, possibly becoming transformed into a portion of the woody branch by an alteration in its structure. This stem is always more slender than was the original ovary. A whole group of young leaves of abnormal shape (long and narrow) may grow out of the flower in this manner. The elongation to form a side branch is sometimes partly above and partly below the ovary, as may be seen from plate 29. It may also be seen from this figure, though not clearly, that the lower flowers on the main stem were normal and have dropped off leaving the growing ovaries behind. Some of these afterward developed into large capsules.

The plant in 1909 which produced only virescent flowers, wilted and died about August 10th for no assignable cause, while the other plants continued to bloom long afterwards. It seemed as though the production of virescent flowers was equivalent to seed production in the physiology of the plant,

and was therefore followed by drying up and death such as occurs with normal plants later in the season. The plant in the offspring of No. 47, showing virescence, was not observed to have virescent flowers until September 29, when the blooming season was nearly past. On this plant the ends of all the branches bore only virescent flowers, while farther down the branches normal seed capsules had been produced. Virescence therefore developd in all the flowers simultaneously but only appeared at the end of the season. The virescent flowers on this plant became fairly stout branches, in some cases even possessing internodes.

In the virescent flowers there was no departure from the normal number of parts, but when leaves developed within these they exhibited no regularity in number or arrangement, though always growing out from just within the cycle of the petals.

DeVries refers to what appears to be a similar case in *Oenothera subovata* (1909, p. 423), but does not describe it. He has also described a different type of virescence which is pathological in nature, due to the attacks of certain parasites. Masters (1869) gives a list of cases of virescence (p. 338) in which he includes Oenothera, with the suggestion that it might perhaps better be called frondescence or phyllody. On p. 252 of the work referred to, cases of frondescence or virescence of petals in *Oenothera striata* are cited.

Polymery of the Flowers.—A number of cases of flowers with an increased number of parts were observed in the cultures of 1909 and 1910. No special effort was made to find them all, but they were recorded as they happened to be observed by myself or my assistant. Masters, on p. 44 of the work above cited, refers to species of Oenothera as exhibiting synanthy. Many of the cases of polyphylly in flowers of Oenothera, to be described shortly, are due to synanthy, as I shall show. Certain other cases will require a different explanation.

DeVries (1909, pp. 472, 482) has recorded a number of cases of polymery from his cultures and in the field at

Hilversum. I will add some observations which extend the range of variation in number of parts, and shall then suggest what appears to me a probable explanation of the phenomenon as it occurs in most of the races of Oenothera. All the cases in 1909, with the exception of two, occurred in hybrid *O. Lamarckiana* from various sources, and these two were *O. brevistylis* plants from a cross with *O. Lamarckiana.* Whether this is of significance as indicating greater variability in plants derived from a cross, I cannot say, but it seems not improbable that this is the case. Using signs for the flower parts I shall now give the formulæ for the flowers recorded in 1909, in which K=sepal, C=petal, S=stamen, N=stigma lobe.

1	$K_7C_?S_{15}N_{13}$	6	$K_5C_5S_{10}N$
2	$K_6C_6S_{12}N_{11}$	7	$K_6C_6S_{12}N_9$
3	$K_7C_7S_{16}N_{13}$	8	$K_7C_7S_{14}N_9$
4	$K_5+C_7S_{14}N_{12}$	9	$K_7C_7S_{16}N^{1}_{7+5}$
5	$K_3C_3S_6N$	10	$K_3C_3S_6N$
		11	$K_3C_3S_6N$

This list of flowers, while shorter than that of DeVries, extends in both directions the range of variations observed by him. The highest number of stamens recorded by DeVries in a flower is 14, while I observed one remarkable flower with 16 stamens and two styles which were separate at the top, in contact below, but terete and easily pulled apart. The hypanthium was also considerably flattened and much thicker than usual and even had a longitudinal groove down its center. This flower gave me the clue to the explanation of these cases of polymery.

DeVries states (1909, p. 483) in regard to his cultures of *O. Lamarckiana* forms, that "trimerous flowers are certainly not present," but he has observed them, though very rarely, in *O. biennis* and in hybrid cultures. In the season of 1909 I observed three such cases, in *O. Lamarckiana* of various descent. The flowers were normal in every way except that the parts were in threes, which made the flowers smaller, though the individual organs were not reduced in size.

[1] This flower had two independent stigmas, and styles which were merely in contact in the calyx tube.

Regarding the explanation of these cases, I soon found that every polymerous flower had two bracts at its base instead of being in the axil of a single bract. These bracts may be entirely independent or they may be more or less coalesced at the base, but they project in opposite directions. (See photograph by DeVries, 1909, p. 472.) In all these cases the ovary and hypanthium are more or less flattened. An examination of the stems which bore these polymerous flowers, disclosed the fact that they exhibited irregularity in the placing of the flowers on the stem, or in other words, variations in phyllotaxy. The flowers and their bracts were not uniformly distributed on the stem, but certain flowers were very close together and others long distances apart. It seems clear that this is the explanation of the phenomenon, which is therefore one of synanthy rather than of polymery. The Anlagen of the flowers are of course laid down and their position determined when the terminal rosette of the stem is very small. Anlagen of successive flowers therefore arise very close together, and if anything leads to variation in their position they will sometimes occur partly in contact or overlapping, giving a flower in which the parts are more or less completely doubled in number. The flower having two independent styles, and the fact that two bracts are always found at the base of polymerous flowers, shows that it must be due to a partial coalescence of primordia, such as I have mentioned. It is interesting to note that flower No. 5, having its parts in threes, was immediately below No. 4, which is heptamerous, and on the same side of the stem. Similarly, the plant bearing flower No. 9 also bore at the same time (Aug. 20) the two trimerous flowers, Nos. 10 and 11. It may also be noticed that in all the polymerous flowers the number of stigma lobes is less than the number of stamens. The same is true of DeVries' records. These polymerous flowers are much larger than the normal owing to the larger number of parts, the parts themselves retaining their usual size, except that the hypanthium and style are stouter, as might be expected, and the filaments are sometimes thicker.

In the season of 1910 a number of additional observations

were made on this subject. The records of these were kept by my assistant, Mr. V. Follenius, during my absence, but I had the opportunity of examining the most interesting cases before the end of the season. Cases of polyphylly or synanthy were found in a much wider range of Oenothera forms than in the previous year. The following is the list:

Formula	Race	Remarks
1..$K_7C_7S_{14}N_{11}$	*O. multiflora*	Two bracts at base of flower.
2..$K_6C_6S_{12}N_8$	*O. multiflora*	Two bracts partly coalesced.
3..$K_6C_6S_{12}N_8$	*O. multiflora*	Two bracts at base.
4..$K_4C_4S_8N_4$) $K_4C_4S_8N_5$)	Race No. 25, from near Liverpool, England	Two perfect tetramerous flowers, with their hypanthia in contact throughout their length and partly fused. Ovaries in contact and partly fused. Two bracts.
5..$K_3C_3S_6N_6$	*O. biennis* × *Lamarckiana*	One bract.
6..$K_3C_3S_6N_4$	" "	One bract.
7..$K_3C_3S_8N_5$	" "(same plant as No. 6)	One bract.
8..$K_6C_6S_{12}N_8$	*O. grandiflora* from Alabama	Hypanthium and ovary flattened. *One* bract.
9..$K_5C_5S_?N_?$	Race 54 × 40	*One* bract.
10..$K_5C_5S_8N_8$	*O. biennis*, Chelsea Physic Garden	Only *one* bract at base of each flower in this race. In one case the bract had two tips, as though resulting from the incomplete coalescence of two bracts.
11..$K_5C_5S_8N_8$	" "	
12..$K_5C_5S_8N_8$	" "	
13..$K_5C_5S_8N_7$	" "	
14..$K_5C_5S_8N_5$	" "	
15..$K_5C_5S_8N_5$	" "	
16..$K_5C_5S_8N_5$	" "	
17..$K_5C_5S_8N_5$	" "	
18..$K_5C_5S_8N_5$	" "	
19..$K_5{}^1C_4S_9N_4$	" "	[1] Two sepals of normal width, 3 narrower, occupying about the width of the other two.

The case of No. 4, in which two tetramerous flowers were found, each with its bract, and only partly fused by their ovaries and hypanthia, is particularly instructive and confirmatory of my explanation. One hexamerous (No. 8) and one pentamerous (No. 9) flower, however, had each but *one* bract at its base, as well as the three trimerous flowers in the race *O. biennis* × *O. Lamarckiana.* It is therefore prob-

able that the latter are real cases of polyphylly and not of synanthy, in which, instead of the partial coalescence of two primordia or their failure to separate, there is a variation in the division of the Anlagen of the various cycles of organs in the flower, resulting in a flower having a larger or smaller number of parts than normal. When, as in flowers 10–18, the androecium is normal while the calyx and corolla show an increase of parts, this may be considered to be due to polyphylly rather than synanthy.

The ten pentamerous flowers in *O. biennis* from the Chelsea Physic Garden were found in a culture of 33 plants, and careful search would doubtless have revealed further cases. Evidently the phenomenon is relatively common in this race.[1] The characters of the race are very constant and are remarkably different from those of any other race of *O. biennis* I have seen. They will be described elsewhere. These pentamerous flowers had invariably but one bract at their base, which would indicate that the phenomenon of pentamery is here due to polyphylly rather than to synanthy, although the fact that one of these basal bracts had a double tip might be considered to favor the interpretation of this also as due to synanthy.

It would seem therefore that while most of these are cases of synanthy, or coalescence of two primordia, the trimerous flowers and also evidently some at least of the other cases with only one bract at base, are real instances of polyphylly, due to variations in the divisions which the primordia of a flower normally undergo.

My conception of the process of synanthy is that, owing to variations in phyllotaxy, two independent flower primordia become so closely approximated that they partly coalesce, and develop harmoniously into a single flower in a somewhat similar fashion to the growth of a plant chimera (sectorial chimera) as described by Baur and by Winkler.

TRICOTYLY.—A number of cases of tricotyly and other abnormalities of the cotyledons have been observed in my

[1] Penzig (1890) states that in *O. biennis* pentamerous flowers are common, the number of "carpels" often running up to 9.

germinating pots each year. They are particularly common in *O. gigas,* but no record of them has been kept.

VARIEGATION OF LEAVES.—Yellowish areas not infrequently appear on the rosette leaves, particularly in the English Oenotheras. One striking case of what was evidently a sectorial chimera according to Baur's (1909) terminology, occurred in a culture of 55 plants very closely resembling *O. Lamarckiana,* but having larger rosettes with rather broader leaves. They constituted the second pure generation from seeds of a plant near Liverpool, England. The green areas on the leaves in this plant are contrasted with areas which are yellowish white, showing a complete absence of chloroplasts. It will be seen that in several leaves the line between white and green tissue passes down the midrib, while one or two leaves exhibit patches of white adjoining the midrib. Plate 31, from a photograph taken June 30, 1909, shows the partly developed rosette. The leaves arising from one side of the stem are wholly white, those on the opposite side are mostly pure green, while several others are green on one-half and white on the other. A few areas of pale green, owing to partial absence of chlorophyll, were also observed. The white areas were of course unable to nourish themselves, and continually died away. The plant never formed a shoot, but died before the end of summer, notwithstanding the most careful treatment. The plants of the previous generation gave no indication of such a condition, which therefore appeared suddenly in this individual, and appeared, moreover, from a seed of a plant which was normal green throughout. Presumably one side of the young growing point was without chloroplasts, but just how this condition came about is at present a matter of conjecture.

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EXPLANATION OF PLATES.

Plate 29.—Plant belonging to a race known as *Oenothera multiflora*, originally derived from the English coast near Liverpool. All but the earliest flowers are virescent or frondescent.

Plate 30.—1, Virescent buds photographed natural size, showing the peculiar baggy appearance of the calyx. 2, Virescent buds natural size, opened to show the small petals, tapering pubescent style and (buds on the left) leaves growing out from the interior.

Plate 31.—Sectorial chimera, in which the leaves on one side of the rosette are lacking in chloroplasts. In certain cases half the leaf is white and half green.

VIRESCENCE OF OENOTHERA.

VIRESCENCE OF OENOTHERA.

CHIMERA OF OENOTHERA.

BOTRYTIS ON CHRYSANTHEMUMS.

BOTRYTIS AS A PARASITE UPON CHRYSANTHEMUMS AND POINSETTIAS.*

BY PERLEY SPAULDING.

During the month of November, 1904, one of the smaller greenhouses at the Missouri Botanical Garden was devoted entirely to chrysanthemum plants which were just beginning to come into bloom. Because of lack of room they were badly crowded together, but especial pains were taken to have the conditions as favorable as possible for the plants. The taller ones, located in the middle of the house, came into bloom first, and it was noted almost at once that the flowers were affected by some disease which attacked the petals. The trouble soon spread to the lower plants as they came into bloom, and the disease was very shortly scattered over the house. So far as observed, the trouble always first appeared on the petals. The diseased flowers were picked as soon as they were seen to be affected, so that the fungus did not have a chance to infest the other portions of the plants; for this reason it cannot be said that the fungus might not have attacked other parts of the plants if it had been left to run its course.

The disease first appeared as tiny, watery, discolored spots, looking as if the petals had been pricked with a needle; this, of course, showed much plainer on the white flowers than on the colored ones (pl. 32, above). These spots were generally more or less numerous on single heads, and even on individual petals; they were often located only on one side of the head, showing that the infection had come from some point on that side of the plant. They very rapidly grew larger, and by the time the affected spot extended over a fourth of the petal, the diseased tissues wilted and dried up. Naturally the tips of the petals were first attacked,

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and they died down to the base as the disease progressed in its course. Very soon after they began to dry, a grayish brown velvety growth appeared upon them (pl. 32, below). This was at once perceived to be composed of the fruiting bodies belonging to *Botrytis vulgaris.* These fruiting bodies were formed within about two days after the beginning of the attack. If allowed to run its course, the disease invariably attacked every ray of the affected head, and in the last stages of disease the affected head was simply a mass of dried rays hanging from the stem, and permeated with the mycelium of the fungus, while all over the outside were the fruiting bodies crowded thickly together.

After the first few flowers became affected, the disease spread very rapidly and caused considerable damage. Many of the finest blooms had to be removed as soon as they opened, thus defeating the purpose for which they were raised. The fungus, so far as could be determined, seemed to exhibit no partiality toward any one variety and counts showed that no one color of blossom was more often attacked than another. During the next two years, at about the same period, in the growth of chrysanthemum plants exhibited by the Botanical Garden, the disease has occurred in varying intensity. The first season it started earlier in the development of the flowers than it has since done, and for that reason, caused a greater amount of damage than at any other time. During the months of November and December, 1906, a Botrytis disease of "poinsettias" *(Euphorbia pulcherrima)* was noted in the same house in which two years before had occurred the above mentioned Botrytis rot of chrysanthemum petals. In this case the fungus attacked the slightly projecting angles on either side of the leaves. It did not attack the red, bright colored leaves at the top of the plants, but only the green, broad leaves which grow along the stem below the red ones. The disease first appeared as a slightly deadened area at the very tip of the angles, which showed beneath tiny white drops of the hardened juice along the larger veins. The fungus seems to attack the tissues in such a way that the white juice of the plant finds exits and exudes

through these weakened spots, hardening quickly upon being exposed to the air. These small, hardened drops of juice seem to be very characteristic of this disease upon this particular plant, it being found to occur constantly only in connection with this trouble; the very earliest stages of the disease could be found only by looking at the under sides of the leaves, and noting the presence of this dried juice at the angles of the leaves. In the earliest stages the leaf tissues upon the upper side gave little or no indication of being diseased, although there usually was a slight yellowish discoloration. As the disease progresses the affected area becomes larger and the extreme tips of the affected angles wither and become discolored; this dying of the tissues progresses toward the mid rib of the leaf as the fungus extends its field of action. In no case, however, did the Botrytis disease alone seem to extend over the whole area of the leaf. On the other hand, after the disease had progressed until it involved about one-fourth the area of the leaf, the effect seemed to be communicated to the petiole, and the leaf was prematurely shed. In this way considerable damage was done by the fungus, since the badly affected plants consisted only of a bare stem surmounted by the broad whorl of red leaves at the top. The absence of the green leaves greatly marred the plants for exhibition purposes. About two days after a leaf is first attacked, the characteristic fruiting bodies of Botrytis are formed in thick groups, clothing the under surface of the affected area.

In the same greenhouse a number of plants of *Primula obconica grandiflora* were found to have the lower leaves also diseased by Botrytis. These plants were in very poor condition, being very short-stemmed, so that the lower leaves lay flat on the surface of the soil, thus giving the fungus the best of opportunities for attack. In this case the affected leaves finally entirely succumbed to the disease, and it even spread from the diseased ones on to the adjacent healthy ones.

These instances indicate the parasitism of Botrytis upon the leaves and petals of the above mentioned plants. No ex-

periments were made to prove the parasitism of the fungus, but considering the present state of our knowledge of this very point, we may well consider this fungus one of the very worst, under certain conditions, with which the gardener has to contend. The writer has seen little or no mention of diseases caused by Botrytis in this country, although this class of trouble seems to be well known upon the Continent. Because of the very slight mention which has been made of diseases of this kind, it has seemed advisable to give a rather extended account of the above cases as noted by the writer.

EXPLANATION OF PLATE.

Plate 32.—Chrysanthemums affected by Botrytis. Above, an early stage of the disease in which some flowers are spotted and a very few bear the fruiting fungus. Below, a later stage, in which the fungus is in full fruit.

FUNGI OF CLAY MINES.*

BY PERLEY SPAULDING.

During the fall of 1906 and the spring of 1907, a number of clay mines in the western part of the city of St. Louis were visited for the purpose of determining the species of fungi occurring upon the timbers. These mines are entered by vertical shafts descending from 75 to 125 feet from the surface. From this entrance shaft galleries run horizontally, or nearly so, for distances varying from a half mile to nearly a mile, and numerous doors are usually located at various places along these galleries. Considerable water seeps into the tunnels from overhead, the amount varying at different seasons of the year, but usually being greater during the winter and spring. Immediately after leaving the foot of the vertical shaft one could detect no light whatever.

Along the galleries of these mines many timbers are used to prevent the caving-in of the roof. The timbers in deep coal mines are placed with considerable care, and are fitted together carefully, because of the great weight which they bear and the permanent nature of many of the galleries. In coal mines the tops of the braces standing each side of the tunnel are notched in such a way that the weight cannot possibly cause the cross piece to slide, or the notches may be cut in the end of the cross pieces. In either case the two timbers are fitted quite accurately together. At this point most of the decay occurs, and the set of timbers has to be removed because of a comparatively small defect.

In the clay mines, on the other hand, the timbers are intended only to prevent the caving-in of the earth immediately above the galleries. The timbers never prevent the settling of the large body of soil over the mine for one or two feet. In fact, a mined area could be detected by an

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expert by the dead trees standing on the surface which were killed by the settling earth pulling the roots in two. These timbers were light compared with those used in coal mining, and were rather carelessly placed in position. The top cross pieces were simple flat pieces of planking which were wedged in place on the supports. The rotting usually occurred in the oak supports. Because the weight sustained is not excessive the timbers rot practically throughout their diameter and for a considerable distance in length before they must be replaced. The clay mines are worked for a number of years, and the timbers must be renewed several times, as they last only about two to three years ordinarily. The timbers used were oaks of various species, and southern pine. The oak pieces were used as braces at the sides, while the cross pieces overhead were of pine. The oak pieces were usually from 6 to 9 inches in diameter and did not have the bark removed, while the pine pieces were sawn mostly from heartwood, and were usually 2 to 3 inches in thickness, and about a foot in width. The pine was used for the overhead timbering because of its greater resistance to rot, it being in direct contact with the soil. All of the timber used was wholly untreated, with the exception of being cut in the proper sizes and shapes for use. It is shipped from outside the city, and is usually somewhat seasoned by the time it is placed in the mine, although little or no attention is given to this detail.

The fungi growing upon the timbers in these mines were situated under very abnormal conditions. There was absolutely no light, and the air of the mines was noticeably moister than that out of doors. The temperature never goes down to freezing, and is very uniform, ordinarily being cool enough so that one may wear a coat with comfort even in the hottest weather. In most of the galleries the sides and top were fairly moist, but not dripping, while in others the moisture was very noticeable, there even being small rivulets of water running along the tramway to the lowest parts of the mine. In all cases the water seeping into the mine had to be pumped out to prevent its filling the galleries.

Quite a number of wood-inhabiting fungi were found to be fruiting in a normal manner, but there were evidently others which were unable to form any recognizable sporophores. Of these latter, one was very abundant, forming dense rounded masses of white mycelium which were so full of water that they collapsed when taken in the hand. Every place where touched immediately assumed a water-soaked appearance. Still another formed black mats of coarse mycelium upon the outer surface of the timbers.

Somewhat to the writer's surprise, the fungi found upon the timbers in these mines were not those which are seen most commonly in the forests of the immediate vicinity of the mines but in a number of cases at least, were comparatively rare ones.

In one of the mines visited by far the most abundant fungus noted was a bright red one, which usually grew in the shape of an inverted cone, attached upon one side or at its apex. It was also present in the other mines, but in lesser numbers. This was identified as *Merulius rubellus* Peck. It was present on over one half of the upright oak timbers, and must be quite destructive, as the timbers last ordinarily but two to three years. Sometimes there were dozens of the sporophores on a single timber, and sometimes but one or two. Usually they were scattered, but they also occurred in large imbricated masses. The young stages were simply rounded knobs of reddish mycelium, of greater or less regularity. The presence of this fungus as the most abundant one in the mine is somewhat anomalous in view of its evident rarity in the forests of the vicinity. It has never been found by the writer anywhere in the vicinity, although he has collected quite carefully for several years in that locality. Glatfelter,[1] however, mentions finding it once, about one hundred miles away.

A single fungus was found occurring upon the pine cross pieces in considerable abundance; it grew in a resupinate form, and was identified as *Fomes annosus* Fr. This is known to be especially destructive to coniferous timber in

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[1] Glatfelter, N. M. Trans. Acad. Sci. St. Louis. **16**: 81. 1906.

Europe, and is not unknown in America. In most localities, however, it is not common. Its natural habitat is upon the roots of various coniferous trees, upon which it produces the so-called "root-rot". The conditions in the mine would seem to be very favorable for this fungus. It was practically the only one which was found occurring upon the pine timbers.

A considerable number of different species were found growing upon the oak timbers. A single sporophore of *Fomes applanatus* (Pers.) Wallr. was seen which came from the mine. It was perfectly normal in every way.

Lenzites betulina (L.) Fr. was found somewhat sparingly upon the oak timbers, and in most cases was normal in appearance.

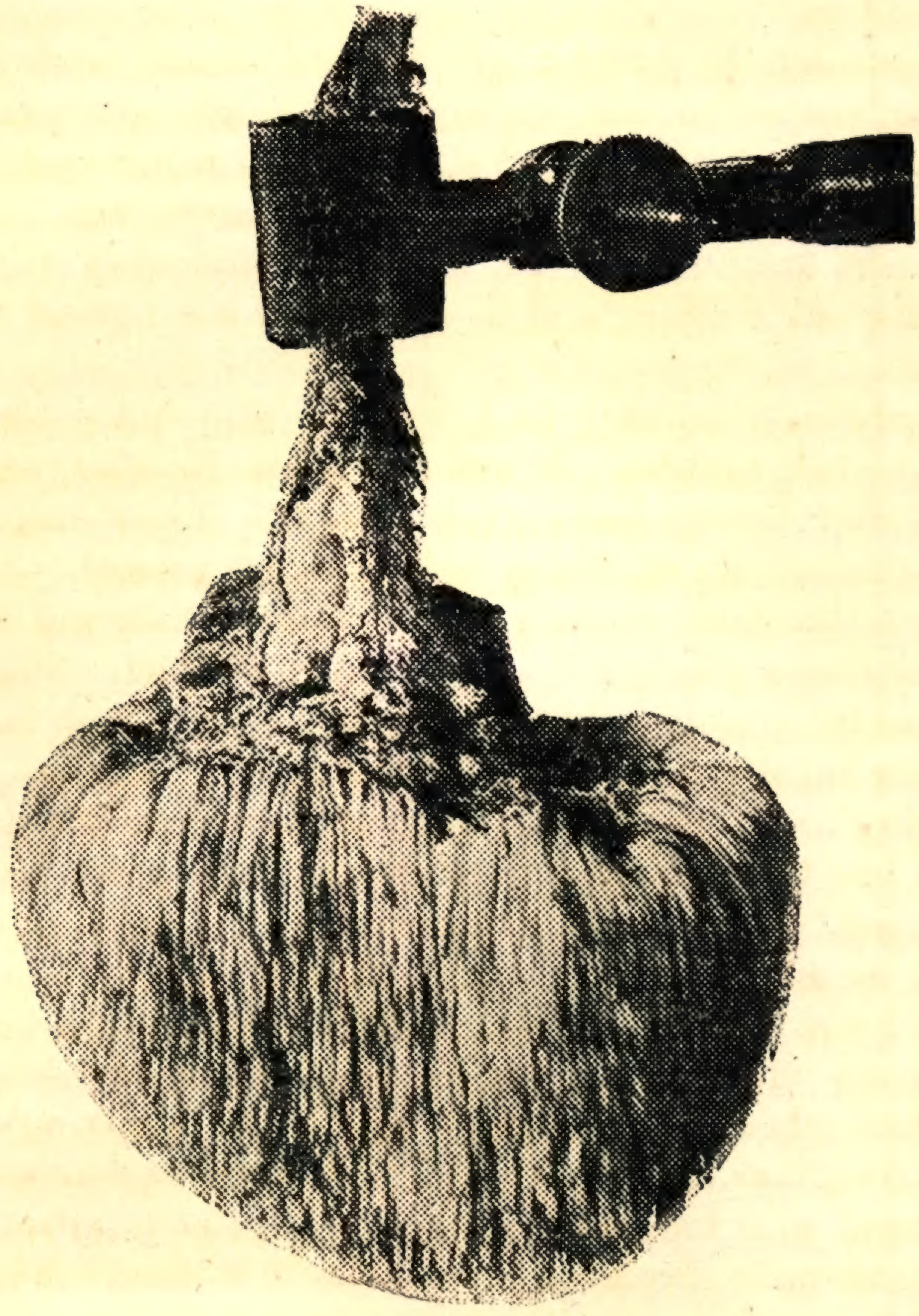
Polystictus versicolor (L.) Fr. was also quite plentiful upon the oak timbers. It was very white in color, and did not exhibit the glistening zones on the upper surface at all distinctly, but otherwise seemed to be normal.

One of the more common fungi upon the oak was *Merulius lacrymans* (Jacq.) Fr. var. *verucifer* Quel. This was noted as being quite frequent in some of the mines, but was not seen in others; it is one of the most destructive fungi occurring in the mines, as a large proportion of the timbers which had been removed were affected with a dry rot such as is caused by the action of this fungus. A rotted stick six inches in diameter when struck sharply upon the ground would break in two very easily, thus showing the extreme weakening effect of this fungus upon the wood tissues.

Several other species of fungi were found occurring rather sparingly upon the oak timbers. *Stereum spadiceum* Fr. was found in a few cases. *Bulgaria inquinans* (Pers.) Fr. was found upon several of the timbers. It occurred in considerable number upon those timbers where it was found at all.

Hydnum Erinaceus Bull. of a rather peculiar type was found hanging from the oak timbers. It grew in the form of a rounded mass, hanging pendant from the lower end of a stout stem several inches in length, and about an inch

in thickness (f. 1). Only two or three specimens of this fungus were found and they were all of the same type. *Hydnum coralloides* Scop. was found growing about normally upon a number of the timbers. *Hydnum artocreas* Berk. was found somewhat sparingly upon the bark of a



1. SPOROPHORE OF HYDNUM ERINACEUS FROM CLAY MINE, × ⅔.

few of the oak timbers in a single mine. This fungus is apparently rare in this country.

A number of edible fungi were also found growing in the mines. Several groups of sporophores of *Coprinus atramentarius* (Bull.) Fr. were found growing upon the clay at the

side of the galleries, and also overhead. They were normal, except that the stems were somewhat longer than usual.

Mules are used quite extensively in the mines to pull the cars along the tramways, and a considerable amount of manure accumulates, which is thrown to one side in some abandoned gallery. In all of the mines entered edible mushrooms of the *Agaricus* type were found growing upon this manure. One found in several mines has been provisionally identified as *Agaricus placomyces* Peck. The men were very well aware that these were indeed "mushrooms", and it was difficult to find enough mature specimens for the purpose of identification, because the men kept them picked closely and took them home to be cooked.

The peculiar feature of the growth of these fungi under such abnormal conditions was that so many of them grew in a nearly normal manner. As above mentioned, masses of mycelium of a number of other forms were very common, but they seemed to be unable to form perfect fruiting bodies. Experiments of the writer in cultivating the wood-rotting fungi for several years past have resulted in the production of perfectly normal fruiting bodies with but a single species, *Schizophyllum commune* Fr. Its absence in the mines was especially conspicuous, not a fruiting body of it being seen, except in one mine which seemed to contain more moisture than some of the others. This fungus seems to tolerate a higher degree of humidity in the air during the formation of perfect fruiting bodies than do most of the wood-inhabiting forms. In this mine the sporophores were numerous, but were somewhat abnormal. They were large, very pubescent, the gills were very wavy in outline, and many were suspended, being pendant at the center from a sort of stalk similar to the specimens of *Hydnum Erinaceus*. In some cases the entire fruiting body was composed of branched *Clavaria*-like growths, showing that the moisture was almost too great for normal sporophore formation. *Polyporus gilvus* Schwein. is exceeedingly common in the forests of that locality, but not a normal fruiting body was found in the mines. A single mass of brown mycelium, however, was

found which very probably was an abortive sporophore of this species. From its known parasitism of roots of living trees one would expect to find *Armillaria mellea* Vahl. in the mines, but no specimens of it were noted, although it is not uncommon in the vicinity upon rotting roots and stumps.

The writer is indebted to Prof. Chas. H. Peck for identifying many of the fungi mentioned above.